

ANNUAL REVIEW OF
ENTOMOLOGY

EDWARD A. STEINHAUS, *Editor*
University of California

RAY F. SMITH, *Editor*
University of California

VOLUME 5

1960

PUBLISHED BY
ANNUAL REVIEWS, INC.
IN CO-OPERATION WITH THE
ENTOMOLOGICAL SOCIETY OF AMERICA

ANNUAL REVIEWS, INC.
PALO ALTO, CALIFORNIA

ANNUAL REVIEWS, INC.
PALO ALTO, CALIFORNIA, U.S.A.

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Library of Congress Catalog Card Number: A56-5750

FOREIGN AGENCY
Maruzen Company, Limited, 6
Tori-Nichome Nihonbashi, Tokyo

PRINTED AND BOUND IN THE UNITED STATES OF AMERICA BY
THE GEORGE BANTA COMPANY, INC.

PREFACE

With the completion of this volume of the *Annual Review of Entomology*, one hundred and two summaries and analyses of entomological progress have been presented during the past five years. Although several of these articles have covered the same general area of knowledge, no two have had exactly the same subject and approach. It will be the continued policy of the Editorial Committee to select each year a number of such specific subjects rather than to attempt to divide entomology into several arbitrary compartments which are reviewed in every volume. The more active areas of research will be selected for review most often (probably every year), while the less active fields will be brought up to date as developments require. It is our hope that the mosaic these reviews present over a period of years is a true and well-rounded treatment of the progress in the many diverse branches of our field of science.

We greatly appreciate the constructive criticism and many suggestions that have come from readers of these volumes. The task of making the selection of review subjects and reviewers is not easy (see Preface to Volume 3) and we actively solicit your advice as to authors and topics.

Success in a review publication depends in great measure on the co-operation, skill, and knowledge of its reviewers. To the one hundred and twenty-two authors and co-authors from ten different countries who have contributed to our first five volumes, we wish to extend our warmest thanks and sincere appreciation. The role of the reviewers becomes more important with each increment to our already voluminous literature. At the same time, the difficulties of the reviewers are multiplying with these increases in the literature and the fragmentation of the field into minute specialties. Authoritative and scholarly reviews by talented and experienced researchers are a necessity in the maintenance of progress and perspective in entomology as well as other sciences.

Readers may note that beginning with this volume, Ray F. Smith, formerly Associate Editor, joins the previous Editor as a full Co-Editor. Anyone who knows the skill and industry of Dr. Smith in undertakings of this kind will realize that this is a natural development and a natural recognition of his talent and services. Together, the Editors will work toward the same goals of excellence and scholarship originally set by the Editorial Committee as an objective of the Review.

The Editors and Editorial Committee wish to express our deepest appreciation to Mrs. Margaret M. Janofsky and Mrs. Betty Schink, who, in spite of extra handicaps imposed by changes in publication dates and the vagaries of the Editors, have skillfully and painstakingly handled many editorial details associated with the publication of this volume. We also wish to thank our printer, the George Banta Company, Inc., for their fine co-operation in the production of this volume.

R.G.	H.H.R.
R.L.M.	R.F.S.
C.B.P.	E.A.S.
C.M.W.	

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INSECT FLIGHT MUSCLES AND THEIR BASIC PHYSIOLOGY^{1,2}

By EDWARD G. BOETTIGER

Department of Zoology, University of Connecticut, Storrs, Connecticut

The flight muscles of insects are extremely well adapted to meet the special requirements of flight. Though our information is limited, it is apparent that in various insect groups different modifications of structure and function have evolved to provide the machinery of flight. Insect flight muscle has been recently reviewed by Pringle (58) as part of a monograph on insect flight. A symposium paper by Boettiger (2) on flight muscles has also appeared. Therefore, this review will attempt an interpretation of the results so far achieved in this field with special emphasis on the most recent work.

To understand the physiological changes necessary to adapt muscle for action in flight, some of the special requirements of flight must be briefly considered. Successful flight involves solving the problems of producing the required aerodynamic forces and of supplying the necessarily large amounts of energy. In order that the aerodynamic forces be maintained, the wings must be driven through a complex cycle. The possession of an exoskeleton with its special mechanical properties has made this complex cycle a relatively easy matter for insects.

In flying insects, especially those with high wing-beat frequency and darting flight, some flight muscles act tonically and others phasically. The tonic muscles hold the articulating parts and insure the proper wing cycle. Except perhaps in insects with low wing-beat frequencies, the tonic muscles do not make adjustments in tension during the short duration of a single cycle. They are used primarily to control the direction and speed of flight. The phasic muscles, acting indirectly in most cases on the wing articulation, furnish the power for flight and, therefore, alter their length at wing-beat frequency, which, in the case of the smallest flies, may be over 1000 times per second (62). It is not always possible to tell morphologically which muscles are phasic. As no physiological work has been done on the tonic flight muscles, this review will be concerned entirely with the phasic type.

The flight path of the wings is determined by the mechanics of the thorax, including the special features of the articulation. The control of these mechanical properties is an important function of both phasic and tonic muscles, for they occupy a good part of the thoracic space and determine the positions of the moving parts. Consequently, though tension in the phasic flight muscles changes during a cycle, it probably never falls to zero.

¹ The survey of the literature pertaining to this review was completed in June, 1959.

² The following abbreviations are used in this review: ATP for adenosine triphosphate; LDH for lactic dehydrogenase.

To achieve the proper aerodynamic force, the wing must be rapidly moved through the cycle. The shorter the wing, the shorter its flight path, and the more times it must reverse in a given period of time to attain the necessary speed. In muscle, whereas the process of activation of the contractile mechanism may be very rapid [Hill (31)], shortening is relatively slow, and the faster stroke can be accomplished only by reducing the amount of shortening. As this involves working at the wrong end of the wing-moving lever, large forces are required. McEnroe (52) found the shortening of the tergosternal muscles of *Sarcophaga bullata* (Parker) during flight to be about 30 μ , or 1 to 2 per cent of rest length. As a consequence, flight muscles do not rapidly shorten. In the desert locust, *Schistocerca gregaria* (Forskål) the average shortening rate is about 40 mm. per sec. [Buchthal *et al.* (11)], while in the fly *S. bullata*, with a wing beat frequency of 180 per sec., it is only 11 mm. per sec. [McEnroe (52)].

Although reducing the shortening solves the problem of rapid wing motion, it presents another. Any series elasticity in the muscle or its connections must be stretched before large external tensions can be generated. Stretching the elastic elements by shortening the contractile is a slow process, so series elasticity must be reduced to a minimum value (11). This is achieved by the attachment of the muscle elements to the skeletal structure by means of tonofibrillae and by decreasing the series elasticity of the muscle fibrils. On the other hand, increasing elastic elements acting in parallel with the active elements is an advantage (11). This passive elasticity resides for the most part within the muscle cell and is not attributable to the sarcolemma. It must either result from elastic elements parallel to nonelastic contractile elements, or the contractile system must be elastic even in the nonactive state [Buchthal & Weis-Fogh (10)].

The rapid movement of the wings is insured by the nature of the loading on the wing muscles. To have maximum speed during shortening, the tension and the load in the muscle should be near maximal at the beginning of the stroke. Flight muscle experiences several types of loading: inertia of the wings, maximal at the beginning of the stroke; damping of the wing movement by the air, maximum near the mid-position where velocity is greatest (work against this load produces the aerodynamic force); elastic loading attributable to straining of the thorax, greatest during the first half of the stroke; elastic loading resulting from stretching the antagonist muscle, increasing throughout the stroke; and finally, changes in the mechanical advantage of the wing lever, the muscle working at greatest disadvantage at the beginning of the stroke.

Though the loading during a cycle has not been directly measured, Weis-Fogh (69) has worked out some aspects of the problem in the locust muscle and supports the conclusion that loading is high at the beginning of the contraction [see also Pringle (58)]. In flies, where the highest frequencies of wing beat are found, Boettiger & Furshpan (9) have described a mechanical feature, the click mechanism, which greatly exaggerates the loading at the beginning of each stroke. The degree to which this mechanism is developed

in different insects is not known, but some such action has been reported in the locust and beetle by Pringle (58). The nature of the relationship between the anterior-notal process and the pleural-wing process makes it highly probable that this type of loading is present to some degree in all insects.

In addition to the adaptations directly involved in achieving the necessary aerodynamic force, flight muscles must be able to deliver larger amounts of energy than any other tissue, for the power used to create aerodynamic forces is greater than for any other physical achievement of animals [Weis-Fogh (68)].

STRUCTURE OF FLIGHT MUSCLES

Tiegs (63), in his extensive study of the anatomy, histology, and development of the flight muscles of Orthoptera, Homoptera, and Diptera, includes a full discussion of earlier work. Pringle (58) has extended the review of 1957 and very few relevant papers have appeared recently. This reviewer will consider mainly those features of the structure of flight muscle that aid in the interpretation of their physiology.

Pipa (53) and Baranowski (1) have studied flight muscle in a number of different insect orders. Depending on the arrangement and size of the fibrils, three principal types of insect muscle have been recognized: (a) lamellar muscle with ribbon-like fibrils of varying width radially arranged in the muscle cell (tubular muscle), (b) microfibrillar muscle with close-packed, nearly cylindrical fibrils averaging 1.5μ unfixed and 0.8μ fixed, and (c) fibrillar muscle with loosely packed, large cylindrical fibrils averaging 3.0μ unfixed and 1.5μ fixed. Although fibrillar muscle has large sarcosomes and is reddish in color because of its high cytochrome content, certain non-fibrillar muscles may have these characteristics to a considerable degree. The most consistent criterion of fibrillar muscle is, therefore, the size and looseness of the fibrils (53). Fibrillar muscle is used only in oscillating systems, and consequently, except for moving the halteres of flies and the tymbals of certain cicadas, it is exclusively a flight muscle. (For a discussion of the microstructure of muscles see p. 20).

Lamellar muscle is found as flight muscle in those insect groups that do not have typical longitudinal flight muscles, namely, Odonata, Blattidae, and Mantidae (53), and according to Tiegs (63) in Thysanura as well. Fibrillar muscle is found only in insects in which one thoracic segment is modified for flight; these insects include Coleoptera, Diptera, Hymenoptera, Hemiptera, and the following families of Homoptera: Cicadellidae, Membracidae, and Aphidae. In addition, Tiegs (63) finds that the Jassidae and Delphacidae, which are closely related to the Cicadellidae, have fibrillar muscle. In *Perkinsiella* (Delphacidae) there are, in addition to the usual mesothoracic flight muscles, a well developed metathoracic flight muscle and an abdominal flight muscle; both are fibrillar (63). Fibrillar muscles can apparently operate in a tandem fashion, one in each of two segments, though the generalization stated by Pipa (53) suggests that this is the exception. At least one species of Cercopidae has small fibrils while others are definitely

fibrillar (63). This family is, therefore, of special interest; more particularly because in all cases so far studied, fibrillar and nonfibrillar muscle have quite different physiological characteristics. Both types of nonfibrillar muscle are classified physiologically as synchronous (2) for, in the flight systems in which they are found, the wings are moved by a powerful twitch of the appropriate muscle group induced in each muscle fiber by a single nerve impulse, resulting in a 1:1 relation between nerve impulse and wing movement. Fibrillar muscle is always asynchronous; the muscle shortening is not a twitch and is not directly correlated (synchronous) with the exciting nerve impulses [Pringle (56); Roeder (61); Baranowski (1)]. Though Pringle (57) has used the term "myogenic" to express the physiological properties of fibrillar muscle, the present author feels that this term should not be extended to include a muscle which depends on the central nervous system for excitation and whose rhythm of contraction is determined primarily by the load. Baranowski (1) has shown that one spittle bug (*Cercopidae*, species unknown) is synchronous. Similarly, representatives of the orders Trichoptera and Mecoptera were found to be synchronous. Phasic flight muscle is nonfibrillar in Orthoptera, Neuroptera, Ephemeroptera, and Lepidoptera according to Pipa (53) and also Roeder (61). Of the few major groups of insects in which no representatives have been studied, only Plecoptera has well-developed flight ability.

Tiegs (63) first recognized the intermediate position of Homoptera in the evolution of flight muscles, as is best demonstrated in his studies on the histogenesis of flight muscle. In Orthoptera, the muscles enlarge by fibril cleavage and, near the last molt, undergo small structural changes necessary to adapt them to use in flight. In highest insects, the flight muscles appear at the metamorphosis from myoblasts, each one apparently producing one fibril. Among the Homoptera, intermediate stages of myogenesis occur. By extending Tiegs' observations and correlating them with new physiological evidence, it should be possible to clarify some major problems, such as the steps by which a transition was made from the more primitive synchronous muscle to the asynchronous type [Pringle (59)].

Regardless of the type of flight muscle, the basic histology is the same as that of other striated muscle [Jordan (38)]. Flight muscles have been studied with the electron microscope but no complete comparative survey has been made (58). Edwards *et al.* (20) have summarized the characteristics of fibrillar muscle as follows: widely-spaced, nonbranching fibrils of large diameter and short sarcomere length, large sarcosomes, little endoplasmic reticulum, abundant tracheoles (intracellular), and dark color. The non-fibrillar muscles differ quantitatively from fibrillar in all these regards but not qualitatively. The smaller amount of endoplasmic reticulum in fibrillar muscle may indicate some special aspects of the coupling process between the muscle fiber membrane and the fibril contractile material, for recent work (37, 54) has suggested that excitation is conducted inwardly to the fibrils by this structure.

Changes in structure during shortening and lengthening have been

studied by Hanson (30), who used phase contrast and polarized-light microscopy in the flight muscle of the fly. At rest length virtually no I band was present and only the Z line was clearly defined. On stretching the muscle slightly, a small I band appeared, and on contraction, induced with ATP, strong contraction bands were found, though length was only 4 per cent below rest length. The small I band was associated with the very small amount of shortening possible in this muscle (52). In all respects, the highly developed fibrillar muscle behaved as rabbit muscle. The stiffness of the muscle was accounted for by the presence of a stroma background in the fibrils which may explain the elongation of the fibrils observed by Hanson (29) under certain conditions.

Innervation.—Except for its general features, the innervation of insect muscle has not been worked out [Hoyle (33)]. A single motor neuron makes many connections with the muscle fiber, so that the full length of the fiber is excited by the motor impulses independently of any conduction process that may be present in the fiber (multiterminal innervation). As is common in invertebrates, more than one axon may innervate a single muscle fiber (polyneuronal innervation). The different axons usually give rise to different transmission processes and different degrees of activation of the contractile substance. On physiological evidence, flight muscle of the locust [Hagiwara & Watanabe (28)] and of flies [McCann (50)] are multiterminal. Though Tiegs (63) describes two axons innervating the flight muscle of Homoptera, Darwin & Pringle (16) find no physiological evidence for polyneuronal innervation in the beetle basalar muscle.

Branching nerve endings are found within the muscle cells in Diptera (63), but it is not clear whether this is a true penetration, with the axon breaking through the sarcolemma. In any case, in Diptera with large fibers and little endoplasmic reticulum, intracellular innervation may be necessary to bring excitation to the fibrils. Edwards, Ruska & DeHarven (19) describe multiterminal neuromuscular junctions in the flight muscle of *Cicada tibicen* Germar. The axon terminals lie in grooves on the surface of the fiber, and beneath the terminal in the fiber a special type of postsynaptic granule is found. This work needs to be extended to include fibrillar muscle. Unfortunately, it was assumed (19) that the tymbal muscle of *C. tibicen* was of the fibrillar type as are those of the cicadas studied by Pringle (57). The tymbal muscle of many cicadas is nonfibrillar (64) and it has been shown that *Magicicada septendecim* (Linnaeus) is synchronous (5). Edwards (17) and Edwards *et al.* (18) have studied with the electron microscope the multiterminal innervation of cockroach abdominal muscle and of the leg of the wasp respectively. Their results should stimulate further work and aid in solving the fine structure of similar innervations in flight muscle.

EXCITATION OF FLIGHT MUSCLE

Information regarding the electrical activity associated with excitation in insects has been obtained mainly from skeletal muscle fibers. In addition to the papers quoted by Hoyle (33), the following recent ones are of in-

terest as their results may apply to some types of flight muscle: Cerf *et al.* (12), McCann *et al.* (51), and Cerf *et al.* (13).

Extending the earlier work of Pringle (56) and Roeder (61) with external electrodes, Boettiger & McCann (7) showed that, in the flight muscle of Diptera and Hymenoptera, the transmembrane response to a single nerve impulse was an all-or-none spike with typical form and reversal of polarization (overshoot). Recording intracellularly from the muscle during tethered flight revealed a very regular discharge of such spikes at a lower frequency than that of the wings. Similar action potentials were found by Hagiwara (27) in locust flight muscle and in cicada tymbal muscle of both fibrillar and nonfibrillar types. McCann (50) has found the same uniformly rising, overshooting spike in the basalar flight muscle of the beetles *Pissodes strobi* (Peck) and *Tenebrio molitor* Linnaeus, and in the longitudinal flight muscle of the hemipteran, *Nezara viridula* (Linnaeus). Darwin & Pringle (16) stimulated the isolated motor nerve of the palm beetle *Oryctes rhinoceros* (Linnaeus), recording from the basalar muscle. A single threshold stimulus produced only a junctional potential. Stimulating at 1 per sec. the potential increased in height (facilitated) with successive stimuli, producing a non-overshooting spike. Since junctional potentials of the facilitating type are found in slow arthropod muscles, Darwin & Pringle (16) suggest that while in Diptera and Hymenoptera a fast innervation (7) is present, in Coleoptera the innervation is of the slow type, and both types of innervation occur in fibrillar muscle.

The original classification of slow and fast nerve fibers referred to the mechanical response, but, as more systems are studied, the distinction is somewhat arbitrary unless applied to two fibers in a single muscle. The facilitating responses of beetle flight muscle described by Darwin & Pringle (16) resemble those found by Furshpan (24) on stimulating the fast fiber to certain Crustacea muscles. Although McCann (50) was able to show typically fast fiber electrical responses in beetle flight muscle, in many penetrations the response to a single stimulus was of the junctional summating type as found by Darwin & Pringle (16). Curiously, the junctional response could be changed progressively into an all-or-none spike response by increasing the intensity of the stimulus (50). This behavior was not found in the other muscles studied and its significance cannot be evaluated without further work.

In all muscles studied by McCann (50), the presence of a junctional component could be demonstrated. In the fly and wasp, though the normal spike is uniformly rising, exposure to ether progressively and reversibly blocks the spike, leaving only the junctional potential. Such potentials are able to summate on repetitive stimulation to generate the spike. In the hemipteran *Nezara*, a definite junctional component was frequently evident as part of the normal action potential, a result probably correlated with the position of the electrode with respect to the junction. In *P. strobi*, there was an interesting alternation between spike and junctional response. Stimulating at 1 per sec., the following sequence of responses was repeated for long periods

of time: normal spike, spike with slowed-falling phase, multiple firing of spikes, multiple firing with reduced number of spikes, and a small junctional response. This behavior is attributed to some special properties of the transmitter mechanism (50). Miniature potentials have been found in unstimulated insect muscle but are usually attributed to injury [Hagiwara (27)]. However, Wakabayashi & Ikeda (65) believe that phylogenetic significance can be ascribed to the presence in flight muscle of these electrical changes which have their origin in the muscle.

Species differences appear to be unusually great in insects, a characteristic that may be caused by the varied ionic composition of their body fluids [Hoyle (32)]. On the basis of electrical response, the excitatory process in *Nezara* resembles more closely that of the Diptera and Hymenoptera than that of the few Coleoptera studied. However, important differences are found in duration and under treatment with various blocking agents. Carbon dioxide, which causes an immediate and reversible disappearance of the muscle resting potential in flies and wasps, has no such effect on *Nezara* or on the beetles studied (50). Attempts to correlate mechanical responses with electrical activity of the muscle membrane are especially difficult in arthropods; see the recent papers of Hoyle & Wiersma (35, 36), and Hoyle (34).

MECHANICAL PROPERTIES OF NONFIBRILLAR FLIGHT MUSCLE

In insects, two different types of muscle have been successfully adapted to meet the severe requirements of flight: a twitch muscle used in the synchronous system and a tetanus muscle in the asynchronous.

The dorsal longitudinal muscle of the locust, *Schistocerca gregaria* (For-skål), is the only twitch-type phasic flight muscle whose mechanical properties have been studied. The results of this work should be widely applicable to all synchronous flight systems. In order to bring their quantitative results into line with those of other muscles, Buchthal, Weis-Fogh, & Rosenflack (11) subtracted from the total force produced by the muscle the very considerable force attributed to passive elastic elements in parallel with the active contractile component. The advantage of such parallel elasticity over an oscillating system is that the energy stored in the elastic elements while the muscle is being stretched may be recovered to aid in moving the wing during the subsequent shortening of the muscle. Without such a storage of energy somewhere in the system, much of the energy used to overcome wing inertia at the beginning of each stroke would be lost. In the locust muscle, Weis-Fogh (68) has determined that parallel elasticity of the muscle accounts for about 50 per cent of the total elastic force and for about 75 per cent in the dragonfly. The parallel elastic elements are as rapid as the contractile elements and dissipate only 20 per cent of the energy.

Under tetanic stimulation, Weis-Fogh (67) found that the isolated locust muscle can shorten to about 50 per cent of rest length and can develop isometrically at rest length the same force per unit cross section as frog muscle. However, at normal temperatures a single tetanus will induce irreversible changes in this twitch muscle. The twitch tension under isometric conditions

approaches the tetanus tension, showing that even a single nerve impulse will very rapidly initiate a full active state in the muscle. The active state declines very rapidly, for if the muscle is allowed to shorten, the active state will decrease considerably before the relatively slow shortening process is over (11). Therefore, the maximum isotonic twitch tension is very much less than the isometric tetanus tension. As the normal contraction is a twitch, in which some shortening is necessary to move the wings, it is apparent that the muscle is always operating below its maximum tension (11).

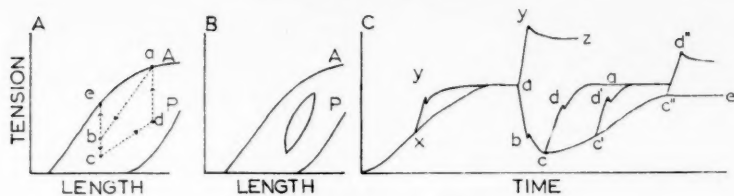
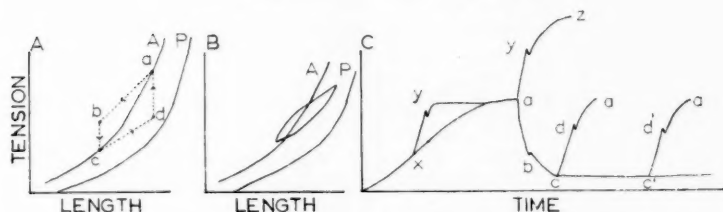
The rapid rise in active state, to oppose the load which is maximal at the beginning of the stroke, and the small amount of shortening allowable if the tension is to remain high—shortening takes time and the active state decreases rapidly—are necessary adaptations in a twitch muscle used in a rapidly oscillating system. Though the work done, each cycle may be small compared to what the muscle is capable of delivering considering its maximum tension and maximum shortening; the high frequency brings the power output up to the maximum that the metabolism can support (11).

The general characteristics of locust flight muscle are surprisingly similar to those of frog muscle. Although the series elastic element was calculated to be double that of frog muscle (the added stiffness in series being a requirement noted above for flight systems), Hill's constants and the maximum isometric force were comparable (11). The study of other nonfibrillar muscle may well reveal some interesting variations in the pattern found in the locust.

MECHANICAL PROPERTIES OF FIBRILLAR FLIGHT MUSCLE

Type 1 fibrillar muscle.—On physiological grounds, at least two types of fibrillar muscle can be distinguished, though the distribution of the two types is not known. The dorsal longitudinal muscle of the bumble bee is the prototype of Type 1 fibrillar muscle. As Hymenoptera and Diptera have similar electrical properties, it may be that these two groups have Type 1 muscle. However, the tension-length curves obtained on the tergosternal muscle of the fly by McEnroe (52) resemble those of Type 2 muscle.

The mechanical response of Type 1 muscle to a single stimulus is a small twitch which will summate with subsequent ones on repetitive stimulation, the tetanus-twitch ratio being as high as in many other arthropod muscles used to maintain tension [Boettiger (2)]. In contrast to the twitch muscle of the locust, one depolarization of the muscle junction does not fully activate the contractile mechanism. The isometric tension-length relation of bee muscle tetanically stimulated is similar in shape to that found in many vertebrate muscles, though the passive stiffness is greater (Figure 1A). The muscle is able to shorten 12 per cent, though *in situ* shortening is mechanically limited to about 3 per cent of rest length (2). The most interesting experiments are those in which the isometrically stimulated muscle is subjected to controlled changes in length [(2) Figure 1A and 1C]. A stretch (α) results in a sharp rise in tension and a fall to a new higher level (α). Such a response is normal for a viscoelastic system and for many muscles.

FIG. 1. Type 1 fibrillar muscle, *Bombus* sp.FIG. 2. Type 2 fibrillar muscle, *Oryctes rhinoceros* (Linnaeus).

- A. Tension-length curve of unstimulated muscle P and stimulated muscle A, showing tension changes induced by rapid length changes
 B. Tension-length loop of freely oscillating muscle
 C. Effect of changes in length on muscle tension

A stretch during the rise of tension following the beginning of stimulation and so before full tension (activation) is attained results in a rise during the stretch (xy) followed by a second rise in tension (ya) more rapid than in the unstretched muscle. The active state increases faster following a stretch.

When the muscle is allowed to shorten rapidly (ab), the tension falls during the shortening, followed by a second fall in tension (bc) not associated with any simultaneous change in length; this second fall is the result of deactivation, a reduction in the tension-sustaining ability of the muscle. The muscle tension then recovers (ce) at about the same rate as the rise of tension on initiation of stimulation, signifying that reactivation is occurring. If the muscle is stretched before reactivation is complete, the rise in tension during stretch is less than expected (cd) and there is a second rapid rise of tension (da) caused by the more rapid attainment of the full active state as a result of the stretch. If stretches are made later, the second rise is less until, when reactivation is complete, it has disappeared altogether ($c''d''$). The isometrically stimulated muscle is maintained at an active state determined by the frequency of stimulation. Only when the muscle is not activated to a level maximum for the stimulating frequency, as during a period of deactivation following a shortening or before activation is complete at the beginning of stimulating, will a stretch result in a delayed second rise in tension and increased rate of activation. Such a system is unstable and when

allowed to shorten with a load, the muscle will oscillate and do external work, represented by the area of loop in Figure 1B, in each cycle against any viscous force impeding its motion (action of the air on the wings to produce aerodynamic force). The frequency and amplitude of the oscillations depend upon loading. When the muscle is driven to oscillate sinusoidally, it may either do work or absorb energy depending on the frequency [Boettiger (2, 3)].

Type 2 fibrillar muscle.—The new preparation introduced by Darwin & Pringle (16), the basalar muscle of the beetle, is more favorable for quantitative study. In the muscle of the beetle *O. rhinoceros* as in the bee muscle, a single stimulus gave rise to a small twitch and the tension increased faster and to a higher level with increasing frequency of stimulation [Boettiger, Machin & Pringle (6), Machin & Pringle (46)].

Work on the beetle muscle soon revealed differences in physiological behavior sufficient to distinguish it as a second type of fibrillar muscle [Machin & Pringle (46)]. The fundamental difference between the two types of fibrillar muscle is that, whereas in the bee muscle (Type 1) the full activation normal to any stimulation frequency is attained at all lengths in response to stimulation, in the beetle the passive length of the muscle determines the degree of activation produced by a given frequency of stimulation [Boettiger (4)], as evidenced below.

The tension-length relationship of the tetanically stimulated muscle is only a little above that of the unstimulated muscle and more or less parallels it without reaching a maximum (Figure 2A); the muscle can shorten very little below the length at which its passive tension is zero [Machin & Pringle (46)]. When the stimulated muscle, contracting isometrically, is allowed to shorten at the proper speed from any point on the tension-length curve, the tension during shortening remains above the isometric curve [(ab) Figure 2A] and after a delay falls to the normal isometric tension characteristic of the shorter length [Boettiger (4, 5)]. Apparently the level of activation of the longer length (*a*) persists for a period during shortening and maintains the tension above the isometric. The delayed effect of the shortening is, as in the bee muscle, a deactivation. However, there is no subsequent slow rise as in the bee, for the muscle after deactivation is at the active-state level normal to its length and frequency of the stimulation (*c*). In any rapid stretch, the length change proceeds (*cd*) at the lower level of activation of the shorter length (*c*) and is then followed by a delayed rise to the new tension and level of activation normal to the longer length (*da*).

A stretch to bee muscle produces no rise in activation unless the active state is below the level normal to the frequency of stimulation [Boettiger (2)]. A partially deactivated bee muscle is reactivated spontaneously within a fraction of a second. In beetle muscle, a stretch always results in a delayed rise in activation [(da, *ys*) Figure 2C and A] shortening in a delayed deactivation (*bc*) from which there is no spontaneous reactivation [Boettiger (4, 5)].

Machin & Pringle (46) applied unique electronic techniques to the study

of the mechanical factors determining frequency of free oscillations of the stimulated beetle basalar muscle and demonstrated that it behaves basically as a mechanically resonating system (Figure 2B). Optimal conditions for maximum work output were determined. The free oscillating behavior of bee and beetle fibrillar muscle was studied in the same apparatus, confirming the existence of two types of fibrillar muscle (46).

The previous explanations of the asynchronous mechanism by Boettiger & Furshpan (8) and Pringle (57) associated changes in activation with sudden changes in muscle loading (quick release and quick stretch). In the beetle muscle, it is apparent that the state of activation depends on the frequency of stimulation and the muscle length. In the bee muscle, the frequency of stimulation has full control of the active state, though a shortening temporarily and partially blocks this, while a stretch speeds reactivation. The change in activation is proportional to the length change and lags by a fixed interval of time. If the muscle is being stretched at the moment when a deactivation following a shortening occurs there is a sharp break in the tension (4) showing that the deactivation process is as fast as Hill (31) has found activation in frog muscle to be.

If one now asks the question how activation and deactivation are tied to muscle length, a very fundamental problem in muscle physiology is being touched upon. Two possible answers have been suggested. One starts with the observation that oscillatory behavior is a fundamental property of contractile systems, the most primitive of which are oscillatory. As muscles were adapted for different functions, e.g. tension production, this property was given up, perhaps, according to Moos & Lorand (45), only inhibited, but still able to be demonstrated under the proper conditions [Pringle (57)]. The demonstration by Goodall (26) and Moos & Lorand (45) that glycerinated psoas fibers of the rabbit will oscillate when treated with ATP favors this idea. The second suggestion considers the oscillatory behavior of insect fibrillar muscle a new property. The excitatory coupling mechanism is thought to be controlled by fiber (membrane) length, length having full control of the active state in Type 2 and only temporary control in Type 1. The delay between length and tension may then be the time required for the effect of the length change to propagate to the contractile material [Boettiger (4, 5)]. If the coupling rather than the contractile mechanisms has been adapted to flight, insect flight muscle presents unique material for study of this little known process.

THE METABOLISM OF INSECT FLIGHT MUSCLES

Results previously reviewed by Pringle (58) have tentatively established the following: (A) Certain insects, e.g. *Diptera* and *Hymenoptera*, use carbohydrate as fuel for flight; others, as the locust, use predominately fat. (B) Little, if any, energy is made available by the usual oxygen debt mechanism irrespective of the fuel used. (C) The glycolytic enzymes are present in the soluble fraction of the muscle homogenate, and the tricarboxylic and H-transport systems are present in the sarcosomes (mitochondria). Oxida-

tive phosphorylation in extracted systems has been demonstrated but not the phosphorylation of arginine or creatine. (D) No extracted systems has approached the QO_2 (μ l. of O consumed by 1 mg. per hr.) of the muscle during flight. (E) Only a small portion of the total phosphorylation is sensitive to dinitrophenol. (F) Flight muscle actomyosin and ATP react as in the vertebrates. (G) Triosephosphate dehydrogenase is not inhibited by iodoacetate. (H) No qualitative biochemical differences have been found to account for the differences between fibrillar and nonfibrillar muscle or carbohydrate and fat-using flight systems.

Recent work has extended these results and provides some basis for interpretation. Zebe (70) found that the resting R.Q. (respiratory quotient) in butterflies was even lower than in the locust, 0.65 to 0.78, and did not change during flight. As in the locust, but not in insects using carbohydrate, the respiration was somewhat raised for at least one hour after the end of flight. Glucose fed to butterflies was converted into fat and there was no evidence that the flight muscle could use carbohydrate directly. There appears to be no correlation between the type of fuel and the type of muscle; the cockroach, *Periplaneta*, uses carbohydrate (42), the locust, fat (39). In view of the contribution made by the fat body (66) one must distinguish between the fuel used by the insect and that used by the muscle. Lipase activity was found by George *et al.* (25) to be greater in dragonfly flight muscle than in that of the locust and less in the bumble bee than in either. The fat body of the moth *Prodenia*, incorporated C^{14} -acetate and small amounts of glucose into long chain fatty acids [Zebe & McShan (73)]. The flight muscle, however, had only 20 per cent of the activity of the fat body in the incorporation of acetate and no activity in the incorporation of glucose. The form in which carbohydrate and fat derivatives are delivered to the flight muscles is not known. The lack of strong fatty acid-oxidase activity in flight muscle suggests that only acetate is used (66).

The oxygen consumption of the resting flight muscle and the activity of its oxidative enzymes are greater than those of leg muscle (40). *In situ* preparations of the flight muscle of the cockroach *Periplaneta*, had three times the QO_2 (μ l. of O consumed by 1 mg. per hr.) of the leg muscle of the grasshopper *Tachycines*. The flight muscle also had greater succinic oxidase activity than the leg muscle [Kubišta (40)]. Because of the high level of cytochrome in *Periplaneta* flight muscle, compared to leg muscle, cyanide was less effective in reducing oxygen consumption [Kubišta (41)]. Whether fibrillar or nonfibrillar, flight muscle has a higher biochemical potential than leg muscle.

Recent evidence suggests that this higher potential is achieved, in part at least, through emphasis on certain pathways which are present but less well developed in other animal tissues. There are many reports that insect muscle, especially flight muscle, has little or no lactic dehydrogenase (LDH); see Pringle (58) for references. The most recent and complete study is that of Zebe & McShan (72) who investigated the flight and leg muscles of representative species belonging to the following groups: Coleoptera, Lepidoptera, Diptera, Hymenoptera, Orthoptera, and Blattidae. In all species, flight

muscle LDH was very low, while glycerophosphate dehydrogenase not bound to structure, GDH-1, was very high. Leg muscles, except for those of *Belostoma* and *Melanoplus*, also showed high GDH-1 and low LDH though the levels were lower, reflecting the lower activity potential of leg muscle. The two exceptions were in insects in which some anaerobic activity might be expected to be useful. *Melanoplus* leg muscle had three times the LDH activity found in the flight muscle of the same insect. The differences in activity of enzymes were not correlated with the type of fuel used. Studying the cytochromes of the fly sarcosomes, Estabrook & Sacktor (21) were able to find spectral differences indicating a deficiency in cytochrome C₁. The difference in LDH activity of insect and vertebrate tissues is clearly related to the more efficient transportation of oxygen via the tracheal system, anaerobic processes being of very minor significance.

Zebe & McShan (72) also demonstrated a particulate-bound glycerophosphate dehydrogenase, GDH-2, that was very active and did not require DPN (diphosphopyridine nucleotide). Such an enzyme, linked in the sarcosomes to the cytochrome system, much as succinic dehydrogenase is linked, has also been described in vertebrate tissue. GDH-2 was found in flight muscles, leg muscles, and in the fat body, especially of the blowfly. Kubišta (42), studying *Periplaneta* flight muscles, concludes that under anaerobic conditions little lactic acid is formed. Most of the glycogen broken down undergoes a dismutation at the triosephosphate stage. The reduced product of this dismutation, glycerophosphate, accumulates in the tissues to an amount equal to one-half the glycogen metabolized. The oxidized products appear as pyruvate, acetate, and possibly as a yet unidentified reduction product of pyruvic acid. In contrast, the femoral muscles of the grasshopper produce considerable amounts of lactic acid. As shown by Zebe & McShan (72), many leg muscles have very low LDH, and the grasshopper is an exception in this regard. The H-acceptor in the oxidative decarboxylation of pyruvate to acetate is unknown. Only one-half as much ATP can be formed by these reactions compared with normal glycolysis, since half the carbohydrate accumulates as glycerophosphate. Probably these effects are not important anaerobic reactions but operate aerobically, as both glycerophosphate and pyruvate can be oxidized by the sarcosomes [Kubišta (43)].

Estabrook & Sacktor (22), and Estabrook *et al.* (23) demonstrated that glycerophosphate is metabolized 10 to 100 times faster by fly sarcosomes than are the intermediates of the tricarboxylic acid cycle. The GDH-2 (particulate-bound glycerophosphate dehydrogenase) responsible for this oxidation was inhibited by versene while certain ions, as Mg, reversed the action of the inhibitor. To explain the very rapid and large increase in metabolism when the muscle becomes active, they suggest that some versene-like substance inhibiting GDH-2 is present, and excitation releases the enzyme from the inhibition through the action of an ion such as Mg. Using glycerophosphate as substrate, Chance & Sacktor (14) were able to construct a system in which the turnover rate of cytochrome C in the fly sarcosomes approached that occurring during flight, a rate much greater than has been achieved with tricarboxylic acid cycle intermediates. Levenbook & Williams (44) have

calculated that cytochrome C turns over once every two wing beat cycles.

As postulated by Zebe (71), glycerophosphate takes part in a cycle by which reduced coenzyme of diphosphopyridine nucleotide, DPNH, transfers its hydrogen to flavoprotein and the cytochrome system. First, dihydroxyacetone is reduced by DPNH with GDH-1 (glycerophosphate dehydrogenase not bound to structure) as enzyme. The resulting glycerophosphate diffuses into the sarcosomes and is oxidized by the action of particulate-bound glycerophosphate dehydrogenase (GDH-2), reducing flavoprotein directly without the necessity for DPN (diphosphopyridine nucleotide). This system has been found in the thoracic muscle of the fly by Estabrook & Sacktor (22), of the locust by Zebe (71), and of the cockroach by Kubišta (42). It is probably not limited to flight muscle, for leg muscles in many insects have high glycerophosphate dehydrogenase activity (72).

In confirmation of earlier work by Gilmour in the locust, Maruyama (47, 48, 49) finds the reaction between bee thoracic muscle actomyosin and ATP to have no unusual features. Estabrook and Sacktor (21), comparing flight muscle sarcosomes with mammalian heart sarcosomes, found the insect had no cytochrome C, but had a new pigment absorbing at 555 $m\mu$. Recently Price & Lewis (55) have studied the phosphorus compounds in blow fly thoracic muscle. Relative to protein, ADP (adenosine diphosphate) and ATP were lower in fly than in vertebrate mitochondria.

The demonstrated peculiarities of insect muscle expressed most intensely in flight muscle are adaptations to insure the most rapid fuel consumption within the special capabilities of the respiratory and circulatory systems of the insect. Oxygen supply is not limiting in many insect muscles, especially flight muscles, and little provision is made for anaerobic process. However, the rapid transportation of metabolites to the muscle and more particularly to the sarcosomes may present a serious problem. The glycerophosphate cycle could function in the transport of hydrogen from soluble enzymes to the electron transport system of the sarcosomes. The source of these hydrogens may be sarcoplasmic reactions such as the monophosphate shunt shown to be present in fly muscle by Chefurka (15) or may be from the fat body in the form of derivatives of either fat or carbohydrate. The fact that no differences have been yet found in the biochemistry of flight muscle correlated with the type of fuel used in flight indicates that structures other than the muscle play an important role in the flight metabolism.

CONCLUSION

Insect flight muscle has acquired some interesting properties in adapting to the requirements of flight. In most cases these involve quantitative rather than qualitative changes in muscle structure, physiology and biochemistry, for many of the features described in insect flight muscle are found in other insect muscles. The relation between active state and muscle length as seen in fibrillar muscle is unique for oscillating systems and is found almost exclusively in flight muscle. However, even in vertebrate muscle, length affects the duration of the active state (60) so that the evolution of fibrillar muscle may not have required a great change in the organization of the muscle cell.

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INSECT MICROMORPHOLOGY¹

BY GEORGE A. EDWARDS

*Division of Laboratories and Research, New York State
Department of Health, Albany, New York*

Inasmuch as there are no general articles on insect micromorphology, this review will attempt to cover most of the studies to date on the fine structure of insect organs and tissues, emphasizing recent findings.

CUTICLE

The fine surface pattern of scales, wings, and integument has given a morphologic basis for interpreting metallic coloration. Three patterns predominate: diffraction grating as in the morpho wing (2, 36, 55), hexagonal cells (15, 16, 36), and microindentations (36). The first and third types have spacings that would produce color; the second, a larger pattern, could be an image of the surface of the cuticle-secreting epidermal cells.

The integument comprises the cuticle, epidermal cells, and their basement membrane. Combined electron microscopic and chemical studies have shown the epicuticle to be nonchitinous and the procuticle to be composed primarily of chitin and protein. Each of these layers may be subdivided structurally according to lamination and micellar orientation, and chemically according to stainability and solubility (62, 64, 67 to 71). Helical pore canals occur in the procuticle. They are probably derived from epidermal cytoplasmic projections around which the procuticle is formed. The epicuticle is formed first, followed by secretion of the procuticle in layers. Sclerotization begins at the interface of epi- and procuticles and spreads inward, not reaching the underlying epidermal cells (63).

At the time of molting, the exposed surface layer of the outer epicuticle of the yellow mealworm (*Tenebrio*) is composed of a wax which melts at 40° C. After molting, a cement layer is secreted first, followed by a finely filamentous layer of wax which melts at 80° C. The waterproofing of the mature pupal cuticle is better than that of the young. It is believed that the waterproofing is due to both the wax and cement, and further that there is a single "outer epicuticle," the main component of which is wax, but which possesses a central zone of bound carbohydrates (47, 48).

Of the two newly described sublayers of the cuticle, the "ecdysial membrane" has been observed in the electron microscope. It is the thin sheet appearing between the new and old cuticles at the time of molting and has differentiated outer and inner sublayers analogous to whole cuticle. It could represent a distinct membrane produced to facilitate molting or could be an incidental change of the laminae of the old endocuticle attributable to premature secretion of sclerotizing compounds for the new cuticle (65, 66).

The epidermal cells in *Rhodnius* undergo considerable morphologic

¹ The survey of the literature pertaining to this review was concluded in May, 1959.

change following injection of the animal with thoracic gland hormone. At rest (following completion of a molt and prior to feeding) the nucleus is small, there is little RNA, and the mitochondria are small, granular, or filamentous (0.15μ diameter). Six hours after injection the mitochondria become swollen (0.28μ), and after 24 hr. they are more swollen and rounded. Similar changes occur after normal feeding. Two days after feeding, RNA accumulates in the cytoplasm, the mitochondria are increased in number, and the nuclei and nucleoli are enlarged. Nine days after feeding, at the height of cuticle formation, the dense ergastoplasm practically obscures the other organelles. The epidermal changes do not occur if the larva is decapitated shortly after feeding since the presence of the brain factor is necessary for the activity of the thoracic gland, the hormone of which, ecdyson, acts directly upon the epidermis and other tissues (87).

SENSILLA

Basiconic sensory pegs, permeable at one end, may number 500 on one antennal flagellum of the male and 700 on a flagellum of a female grasshopper (75). Each peg is associated with approximately five sensory cells. The cuticular wall of the peg is produced by the trichogen cell. Invagination of the peg wall from its distal tip to just above the sensory neurons forms the peg sheath. The lumen between sheath and peg wall is believed to be a vacuolar extension from the trichogen cell and to contain a fluid which may aid in keeping moist the distal nerve processes of the bipolar sensory cells. The nuclei of the nerve cells are larger and rounder than those of adjacent epidermal cells. Pigment granules, common to the epidermal cells, are not seen in the nerve cells. The proximal nerve processes from several cells unite to form a large nerve passing to the central nervous system. The converging distal processes of the nerves enter the cuticular sheath, decrease in diameter toward the top of the peg, but extend the entire length of the peg. Both trichogen and tormogen cells show micromorphologic changes, e.g., nuclear enlargement, during secretion of the cuticular and membranous portions of the peg. After the cuticle is formed, however, these specialized cells appear similar to adjacent epidermal cells. The top of the peg may or may not be lost during molting. Loss of water may occur through the permeable tip, but the quantity involved is small.

The hairs containing sugar receptors in *Phormia* are characterized by two lumina, one continuous with the body of the trichogen, the other containing processes of the bipolar sensory neurons. The fibers terminate at the permeable papillate tip of the hair. From the known sizes of the microstructures of the hair sensillum it has been calculated that 1×10^9 molecules of sucrose can diffuse to the receptor site in 0.1 sec., a sensitivity less than that of olfactory receptors (22).

TRACHEAL SYSTEM

Isolated tracheae and tracheoles have a fundamentally similar structure, including taenidia, down to the finest branches; taenidia may be either helical

or annular, and micropapillae exist on the epicuticular covering of the taenidia (33, 67 to 71, 78). The cuticle of large tracheae was initially thought to comprise a tubular basal membrane, a surface membrane, and enclosed taenidia, representing procuticle and epicuticle—the micellar orientation being parallel with the long axis in the basal membrane and perpendicular to it in the taenidia. This arrangement was believed to result from the action of stress forces during development (62, 64, 71).

Thin sections showed that tracheoles (13, 25, 26, 36), whether passing between cells or indenting them ("intracellular"), are always enclosed within tracheoblast membranes (27, 28, 29, 80). The tracheoblast (tracheal end cell, stellate cell) is generally accepted as an epithelial cell modified for chitinogenesis (4, 29, 56, 57). The tracheal intima is formed by secretion across either membranes of the endoplasmic reticulum or the plasma membrane. The mestracheon is a simple membranous ensheathment of trachea or tracheole (29, 57) or a complex, microvillated ensheathment as in the light organ (4). The tracheoblasts and their cytoplasmic extensions containing tracheoles, together with basement membranes of all cells, form the insect "connecting tissue" (27, 29).

The tracheal intima consists of an epicuticular lining and a taenidial substance. The nonchitinous lining is similar to the cuticulin layer of the epicuticle of the integument. The taenidial component may be homologous with the procuticle (29, 56). Beneath the 12 to 20 μ cuticulin layer is a protein-chitin layer of 24 to 37 μ thickness with axially oriented micelles. The chitin micelles of the taenidial component are tangentially oriented (56). The taenidia, whether helical or annular, could arise by folding of the epicuticle (29) or by axial buckling when the epithelium separates from the old trachea. The taenidial spacing would thus depend upon buckling frequency and the tracheal size upon expansion (57). Tracheoles possess the cuticulin layer, small or no taenidial component, and membranes of tracheoblast extensions (29, 57). The tracheolar structure could be interpreted as due to a buckling frequency different from that of tracheae (57).

EPIDERMIS

The epidermis consists of a monolayer of epithelial cells having a micro-morphology consistent with active secretion. They are bounded peripherally by a subcuticular membranous layer and proximally by polysaccharide, and a basement membrane (60 to 110 μ thick which is continuous with connecting tissue elsewhere (56). The nucleus of the cell is large, is limited by a double membrane, and possesses numerous pores. The cytoplasm is characterized by numerous layers of meandering ergastoplasmic tubules continuous with outer nuclear membrane and plasma membrane. Secretory granules of several sizes and densities occur in the cytoplasmic matrix and within the reticular canaliculi. The subcuticular membranous layer appears to be finely filamentous. The basement membrane material consists of a loose feltwork of fine filaments with an axial period similar to that of basement membranes surrounding nerve and other tissues (79).

MYOCHITIN JUNCTIONS

Myofibrils terminate at extensive Z bands composed of dense filaments and membrane-limited vesicles, which together with the limiting plasma membrane form irregular projections interdigitating with the epidermal cell border. The apposing plasma membranes of muscle cell and epidermal cell are thickened and vesicular, and the interspace contains oriented basement membrane material. Presumably cuticular substance formed within the cell becomes firmly cemented to the basement membrane when the cell breaks down at the point of apposition. Thus tonofibrils consist of cuticular material continuous with the procuticle distally and with the muscle border proximally. Each myofibril appears attached to the cuticle by approximately 12 tonofibrils, and one epidermal cell provides anchorage for approximately 15 myofibrils. The myochitin junction is similar in structure and density to intercellular bars of vertebrates, although the chemical origins are different (79).

MUSCLE

All insect muscles are striated; however, they differ considerably according to their specialized functions. As in the vertebrates, the majority of muscles are of low frequency and possess fibrillar fibers. Some low frequency muscles are afibrillar, and the high frequency flight and tymbal muscles of higher insects are fibrillar. A given low frequency muscle may contain only one type of fiber or both afibrillar and fibrillar. High frequency muscles contain only fibrillar fibers.

Afibrillar (tonic) fibers occur in intersegmental muscles of larvae and in adult visceral and dorsal vessel muscles (12, 30, 35). They are characterized by the arrangement of the contractile material into fields separated at irregular intervals by a sparse endoplasmic reticulum. Mitochondria are small and few in number. The sarcolemma possesses a thick basement membrane. Tracheolization is sparse. The sarcomeric period is long. M and N crossbands are lacking. There is apparently only a single array of myofilaments in these fibers.

The low-frequency fibrillar muscles may be arranged into two groups micromorphologically. The fibers may possess cylindrical myofibrils, as in all leg muscle fibers (24, 26, 27, 30, 32 to 35), or may possess band-shaped myofibrils, as in the flight muscles of a dragonfly (23) and a cockroach (30). In both types of fibrillar fibers the myofilaments are grouped into discrete, often-branching myofibrils, which are usually closely packed. The sarcomeric length varies inversely with the frequency of contraction. M and N crossbands are lacking. The small mitochondria occur generally in pairs at mid-I band level between adjacent myofibrils. The system of tubules and vesicles of the endoplasmic reticulum is continuous transversely from plasma membrane to outer nuclear membrane and longitudinally surrounds the individual myofibrils. Aggregates of vesicles occur at mid-I band and mid-H band levels. Reticular triads at A-I junctional regions are common. The nuclei are generally central. The scalloped sarcolemma possesses a basement membrane of

variable width. The dragonfly flight muscle varies from the above general architecture in that the mitochondria are numerous and large and the paired, unusually large vesicles of the reticulum occur at Z-band level.

The high frequency fibrillar flight muscles have been the most commonly studied (13, 26, 28, 30, 32 to 35, 45, 46, 61). In these the cytolemma is relatively thin, the elements of the endoplasmic reticulum are sparsely distributed (with the exception of the cicada tymbal muscle fibers), and the mitochondria are numerous, large, and filled with cristae. The myofibrils are widely spaced, have short sarcomeric periods, and all crossbands are present except the N. The myofilaments have an axial period of $40\text{ m}\mu$ and each is hollow and linked to its six nearest neighbors by filamentous bridges, except in the H band. Thus, in cross section, the filaments present a hexagonal pattern. There is still dispute as to whether insect myofibrils possess a double array of filaments. The solution of this problem is important in the interpretation of the contractile mechanism since the double array permits a sliding-filament arrangement (52), and a single array favors coiling.

During pupal development of the honey bee, the first muscle fibers of the thorax and a negligible quantity of extractable contractile protein appear two days after pupation. On the third day, myofibrils up to $0.6\text{ }\mu$ in width are observable. By the time of emergence the myofibril width is more than $2\text{ }\mu$. The sarcomeric length (2.0 to $2.4\text{ }\mu$) remains constant during metamorphosis. A and I regions and Z band are visible in early myofibrils. The M band appears only after seven days. The mitochondria change from small rods to large, rounded structures during development. The changes in fibrillar structure agree well with the increase in amount of extractable actomyosin. The mitochondrial enlargement can be related to the activation of the respiratory enzymes, including magnesium-activated apyrase, during the period of emergence (53).

PERIPHERAL NERVES

The fine structure of peripheral nerves has been observed in a dragonfly, cockroach, cicada, and wasp (23, 24, 27, 28). Apparently only a "tunicated" nerve-sheath cell arrangement exists in the insect. The lemnoblast (sheath cell) possesses a thick (up to $200\text{ m}\mu$) basement membrane of circularly oriented fine filaments. The plasma membrane invaginates at one or more points to form the loose, meandering mesaxons. The mesaxon makes three to five loose turns about the axon with no deposition of myelin. The nucleus and cytoplasm of the lemnoblast contain the commonly observed organelles, although the Golgi complex is generally small. A given lemnoblast may enclose more than one axon. In the multiaxonal peripheral nerve branches there is often one large central and many peripheral small axons (sometimes only 100 to $200\text{ m}\mu$ in diameter), each within its mesaxon. Basement membrane material is found within the mesaxonal interspace, often deeply within the lemnoblast (thus equivalent to glia). Even the smallest nerve branches are accompanied by tracheoblasts, the basement membranes of tracheoblast and lemnoblast generally being fused.

NEUROMUSCULAR JUNCTIONS

Synapses between nerve and muscle are generally simpler in the insect than in the vertebrate. The axon of the peripheral nerve contacting the muscle fiber courses longitudinally in a groove formed by the indented plasma membrane of the muscle fiber and capped by merged basement membranes of muscle fiber, lemnoblast, and tracheoblast. In the simplest form, as in wasp leg muscle (27), the final synapse is between axonal and muscle-fiber plasma membranes, separated by an interspace of only 12 μ . No basement membrane material intervenes in the insect synapse. The synapsing axonal terminus contains many mitochondria and myriads of synaptic vesicles. The aposynaptic region of the muscle fiber contains aposynaptic granules, mitochondria, and endoplasmic reticulum. No complex folding of the plasma membrane occurs.

A more complex junction is seen in tymbal and flight muscle (28) of the cicada. In addition to the above-mentioned components, the aposynaptic region of the muscle fiber contains a "rete synapticum" of ramified and layered membranes of the endoplasmic reticulum often continuous with invaginations of the plasma membrane.

Multiterminal innervation has been observed in muscles of a dragonfly (23) and cockroach (24). Up to four synapses, spaced regularly about 15 to 20 μ apart, occur beneath a nerve-tracheole net on the muscle fiber surface. In the cockroach, the adjoining peripheral nerve may contain a large central and several smaller peripheral axons, the innervation being multiaxonal and multijunctional. The individual synapse in a given locale, however, involves only one axon. In the dragonfly a similar innervation may occur, but a given synapse involves two axons, one large and one small, in the synaptic groove. These could represent "fast" and "slow" axons, or inhibitory and stimulatory axons. In either event the micromorphologic picture supports electrophysiologic evidence that a given muscle response may be due to specialization of neuron as well as of muscle fiber.

The known micromorphology of nerve, synapse, and muscle fiber make possible the interpretation of the probable pathway from the origin of nerve-action potential through to muscle-cell response. The variation in quantity and distribution of membranes of the endoplasmic reticulum with specific nerve and muscle types, the membrane separation of the cytoplasm into ionic phases, the continuity of endomembrane systems with plasma membrane, and the junctional aggregation of vesicles and tubules all suggest that the action potential originates in nerve endomembranes, moves along the nerve axon and muscle fiber by plasma membranes, and is then propagated within the muscle cell by the endoplasmic reticulum (73).

GANGLIA—CENTRAL NERVOUS SYSTEM

Thoracic ganglia of *Melanoplus differentialis* (Thomas) show a Golgi complex and mitochondria in the cell body but not in the axons. Neurofibrils in the axons are approximately twice the size of those in the cell body (7). The cerebral macroganglion cell of *Drosophila melanogaster* (Meigen) has a nucleus

of about 5 to 8 μ in diameter; the nucleus of the microganglion cell averages 2.6 to 3.0 μ . The nucleus contains coiled euchromatic threads and a dense, oval chromocenter containing DNA (deoxyribonucleic acid). As prophase advances the chromocenter divides into four pairs of heterochromatic bodies (20).

Ganglia, connectives, and large peripheral nerves are ensheathed by a neural lamella of variable thickness. In cockroach ganglia the lamella appears as a two-layered feltwork of filaments having striations similar in appearance to vertebrate collagen (44). The neural lamellae of ganglia and large nerves of *Rhodnius* appear similar, the filaments having an axial period of 50 to 60 $m\mu$ and diameter of 15 $m\mu$ (76). It seems likely that secretion of basement membrane material is a function of all cells (24, 27, 28, 29), but amoebocytes may be those involved in the formation of the neural lamellae (86).

Both ganglia and nerve-cord connectives of the cockroach have been investigated in the electron microscope (44). In the ganglia there are two types of nerve cells. Dark cells are characterized by a dense cytoplasm due to an even distribution of RNA (ribonucleic acid) granules. Light cells show RNA granules in clumps and relatively empty spaces between organelles. The nucleus of the nerve cell has a homogeneously granular matrix and prominent nucleolus. The plasma membrane is rough-edged, showing invaginations over considerable areas, and often forming pockets in which neuroglial processes are included. The mitochondria, Golgi membranes, and lipochondria are randomly distributed.

Neuroglial cells (sheath cells) are distinguished by nuclei with peripheral clumps of chromatin and by long, membrane-enclosed cytoplasmic processes, which form two to twenty overlapping layers ensheathing the nerve cell and axons. Often the processes indent the nerve cell, much as tracheoblast extensions indent a muscle cell. The cytoplasm, including that of the processes, contains granules, vesicles, and Golgi membranes. The axons in the ganglia vary from 1 to 2 μ in diameter. The larger axons are ensheathed singly, the smaller ones in groups. The axoplasm may show mitochondria, granules, and vesicles. Synapses are axo-axonal, not between axon and cell body. Presynaptic axons are identified by their synaptic vesicles, but not all vesicle-filled axons enter into direct contact with others. Postsynaptic vesicles have not been observed in the axoplasm. Three types of particles occur in the axons: those from 30 to 50 $m\mu$ in diameter are considered as granules, those from 100 to 150 $m\mu$ as vesicles, and others from 120 to 250 $m\mu$ and over, and more dense, are called droplets. The particle types could be related to different transmitter substances. (However, unpublished observations by this reviewer of the same material at higher magnifications show that the dense granules are neurosecretory.)

The tracheal cells, among the nerve cells, resemble neuroglia, but their cytoplasm is less complex. Between the basement membrane and the nerve proper are cuboidal perilemmal cells, characterized by round nuclei, light cytoplasm, large and numerous mitochondria, and a smooth plasma mem-

brane. Both nerve fibers and sheath cells become attenuated in passing through a ganglion. In the connectives, the basement membrane is thin and the perilemmal cells do not form a distinct layer. The arrangement of neuroglial cells and neurons in the connectives more closely resembles that in peripheral nerve than in the ganglia.

OCELLUS

The ocellar photoreceptor cells of the dragonfly (72) are arranged in groups, or retinulae, as in the eye. Part of the distal end of each receptor cell is differentiated as a rhabdomere, composed of a large number of microvilli derived from the cell border. The total ocellar sheath consists of fat-producing epidermal cells, the basement membrane of which merges with that of peripheral pigment cells. The black pigment cells are of two types: the peripheral, which serve as sheath cells of peripheral axons, and the central, which ensheath the first-order axon bundles. In these cells, pigment granules in various stages of development are seen within ergastoplasmic whorls. The white pigment cells extend between the retinula cells, have their nuclei in the subcorneal region, and are facultatively sheath cells of retinula cells and first-order axons. They characteristically contain vacuoles, with dense linings, and appear to have contained crystals, perhaps urate, before sectioning. The retinula cell extends distally between tapetal cells. Proximally it forms the sensory neuron, the branches of which eventually synapse with the ocellar nerve branches. Characteristically its cytoplasm contains rows of mitochondria delimited by endomembranes, extending between the microvillated inner border and the pinocytotically active outer border of the cell, and oriented into a discoidal pattern around the rhabdome. The endoplasmic reticulum in the nuclear area is vesicular, whereas in the region of sensory-axon origin it becomes tubular. The sensory axons contain large, peripheral mitochondria, neurofilaments, and few membranes of the reticulum. Ocellar-nerve axons have smaller, central mitochondria. In all synaptic regions, myriads of synaptic vesicles are present in the presynaptic axon. On some few occasions postsynaptic vesicles have been seen in branches of the ocellar nerve. Neurosecretory granules occur in many axons; indeed, a given axon may contain synaptic vesicles, neurosecretory granules, and neurofilaments in a single locus. Synapses occur along the entire nerve area, i.e., small termini of the ocellar nerve synapse with large sensory axons in the cup area, intermediate sensory with ocellar branches in the central zone, and small, tapered termini of sensory branches with branching giant axons of the ocellar nerve at the base of the ocellus.

COMPOUND EYE

One of the most difficult problems to solve micromorphologically has been that of the insect photoreceptor elements. The ommatidium contains a variable number of retinula cells, whose medial borders are modified to form the rhabdome. In *Drosophila* the retinula cells are radially arranged to form a

cylinder of seven rhabdomeres. The rhabdomeres may be 70 to 125 μ long and the cylinder 17 μ in diameter. In longitudinal section the rhabdomeres show alternating dense membranes and less dense intermembranous regions (17, 88, 89, 90). In cross section the appearance is reticular, and in oblique section it is mixed reticular and lamellar (17, 88, 89, 90). Similar structural relations in the seven rhabdomeres of *Musca* (37) and *Sarcophaga* and the three fused rhabdomeres of *Anax* (43) have led to postulates of several different discoidal or lamellar models that might depict the function of the eye as an analyzer.

A high-resolution electron microscopic study (38) of the structural detail of apposition, superposition, and intermediate types of compound eyes, particularly in *Erebus* and a skipper, has clarified the status of the rhabdome and its relation to the sensory cell. In all types of image-forming eyes, the rhabdomeres consist of closely packed tubular units, from 40 to 120 $m\mu$ in diameter, oriented at right or oblique angles to the long axis of the rhabdome. The tubular units are the microvilli of the retinula-cell border, the membranes of the microvilli often being closely associated with the membranes of the endoplasmic reticulum. Within the microvillus there may occur a fine particulate component. The rhabdomeres are symmetrically and radially grouped around an axial cavity which may contain a loose filamentous network or compact bundles of filaments averaging 15 $m\mu$ in diameter.

In the superposition eye of *Erebus*, the receptor layer is separated from the dioptric. The retinulae show a hexagonal array. The rhabdome comprises seven fused, wedge-shaped rhabdomeres. The microvilli, 60 to 120 $m\mu$ wide, form an extremely regular pattern and contain a granular component in the cytoplasm.

The rhabdome of the skipper comprises eight fused, symmetrically arranged rhabdomeres. The marginal cytoplasm of the retinula cell contains many mitochondria in radially oriented rows delimited by small cross-striated, double membrane-limited structures, designated tentatively as ultra-tracheoles. In addition to the numerous mitochondria and reticular membranes in the cytoplasm of the retinula cell, there occur "fenestrated bodies," of regularly disposed membranes. The origin and function of these bodies is unknown. Intimately surrounding the retinula cells of the skipper are single large tracheae. The tracheoblasts appear to be also the dark pigment-producing cells of this area, their cytoplasm showing numerous scattered pigment granules. The tracheation of the retinula cells of *Erebus* is less regular, in that layers of tracheoles surround each group of retinula cells.

When observed at the level of the dark pigment layer, the sensory axons derived from the retinula cells in *Erebus* occur in bundles of seven to nine each. The entire bundle is ensheathed apparently by a single sheath cell. The axons contain, at this level, filaments, granules, and peripherally disposed mitochondria. The occurrence of more than seven axons indicates that branching of the sensory neurons begins just below the body of the retinula cell.

DORSAL VESSEL

The dorsal vessel of both a hornworm and cockroach (12) consists of a cylindrical monolayer of tonic muscle fibers. The fibers are invested by a thick basement membrane. The myofilaments are arranged in fields extending from small areas to the total width of the fiber. A well-defined endoplasmic reticulum surrounds the bundles of contractile material and is directly connected with the deeply invaginated plasma membrane at Z-line level. The filaments possess a Z line, but apparently lack other cross-striations. Mitochondria are aggregated in outpocketings between the invaginations of the cell border. The nuclei are most generally in intraluminal extensions of the cells. At points of apposition between cells the cytolemmas are modified in a manner similar to complex intercalated discs of vertebrates.

The dorsal diaphragm, also one cell thick, is composed of cells bounded by thick, felted filaments. The cytoplasmic microstructure is that of actively secreting cells (as is the microstructure of the pericardial cells situated immediately dorsad of the vessel) comprising, for example, abundant endoplasmic reticulum, ductuli, and contained secretory granules.

Situated on and near the heart are multiaxonal ganglia; each ganglion is limited by a thick basement membrane, often continuous with the basement membrane of the heart-muscle cells. The enclosed axons possess mitochondria, vesicles, and oftentimes numerous secretory granules. The axonal branches form simple synapses with the muscle and diaphragm cells.

INTESTINE

In the light microscope the striated border of the insect gut appears to be composed of five layers. Electron microscopic studies of the gut in *Melanoplus differentialis* and larval *Malacosoma* sp. (5) show these layers to be arrangements of long microvilli on the luminal side of the epithelial cells. The five layers thus resolve into (a) mitochondria and projections of bases of the microvilli in the cortical cytoplasm, (b) plasma membrane at the border of the cell body, (c) proximal portions of microvilli, (d) twisted regions of microvilli, and (e) tips of microvilli. The microvilli are long, often branched, average 70 to 100 μ in thickness, and have their origins deep within the cortical cytoplasm. The villi are straight immediately without the cell cortex, twisted in the middle portion, and straight again at their periphery. They are but extensions of the plasma membrane of the cell and thus contain cortical cytoplasmic matrix. The microvilli of the grasshopper gut are longer than those of *Malacosoma*.

The bases of the microvilli show branching. There are no basal filaments, but the villi are intimately associated with peripheral vesicles of the endoplasmic reticulum. Spiral filaments appear around and between the microvilli of the grasshopper.

The basal cytoplasm of the epithelial cell in *Malacosoma* is divided into a series of mitochondria-filled, incomplete compartments formed by infoldings of the plasma membrane. The arrangement is thus similar to that of grasshopper Malpighian tubule cells (8), crayfish nephron (6), and cells of the

proximal convoluted tubules of the vertebrate kidney (74). By analogy with the vertebrate it can be assumed that the microvilli add to the free absorptive surface and that the abundant mitochondria in the compartmentalized basal cytoplasm provide energy to facilitate exchange across the passive basement membrane.

PERITROPHIC MEMBRANE

The peritrophic membrane was first observed electron-microscopically in dried and shadowed preparations from the gut of *Dixippus* (51), *Periplaneta*, *Tenebrio*, and *Bombyx mori* (Linnaeus) (49), and in the excrement of larval *Tineola biselliella* (Hummel) (54). From these studies the membrane was conceived to be a layered network of fibers at 90° to each other, the fibers chitinous in nature, and the whole perhaps functioning as a dialyzer. Subsequent studies (31, 50, 58, 60) of other insects showed further that the peritrophic membrane may take several different forms, viz., the "regular fibrillar network" of hexagonal symmetry, the "honeycomb network," and the "irregular meshwork" (60).

The regular fibrillar network is composed of long, straight fibrils averaging 6 to 10 μ in diameter. The hexagonal net is formed by three sets of fibers at 60° to one another. The space between parallel strands averages 0.15 to 0.2 μ . The irregular meshworks are composed of bent fibrils felted together in an amorphous-ground substance. This may be a variant of the first type. The honeycomb arrangement may be an encrustation of the regular fibrils by the amorphous substance. The 60° array of fibrils prevents tearing in any one direction. The fibrils are presumably chitinous, the amorphous substance being proteinaceous. The pattern assumed by the membrane could be a reflection of the surface pattern of the mid-gut surface, indeed could reflect the pattern of the peripheral arrangement of microvilli. The suggestion has been made that the fibrils of the peritrophic membrane are formed by deposition of a secretion onto a surface acting as a hexagonal template, the membrane secondarily delaminating from the surface (60).

SALIVARY GLAND

The abundance of nuclear pores in the cells of the salivary gland of *Drosophila* was noted early (3). The relation of these to a changing nucleo-cytoplasmic exchange mechanism was determined later by observing the progressive modifications of nuclear and cytoplasmic structure during the third-larval stadium (40, 41). In the early third-instar larva the cells of the salivary gland contain scattered mitochondria, vesicles and tubules of the endoplasmic reticulum, and RNA (ribonucleic acid) granules in the cytoplasm. The nuclear membranes are smooth, and the chromatic material is divided into aggregates of dense bands and light interbands respectively indicative of chromomeric and nonchromomeric regions of the chromosomes. The chromonemal strands average 20 to 50 μ . At the middle of the third instar the function of the gland changes from the secretion of saliva to the production of a mucoprotein believed to anchor the puparium to the substrate. PAS-

positive (*p*-aminosalicylic acid) granules appear in the cytoplasm, and lamellae of the endoplasmic reticulum become abundant, particularly near the nucleus. The outer membrane of the nucleus forms blebs, and chromosomal material appears in those regions of the nuclear matrix near the blebs. Indeed the blebs often appear opposite heterochromatic regions of the chromosome. Before puparium formation the blebs decrease in number. It is postulated that the membranes of the endoplasmic reticulum are involved in the formation of both saliva and puparium substance. The blebbing of the nucleus could be related to a high degree of polyteny permitting the localization of DNA (deoxyribonucleic acid) from chromosomal material and its transport into, or effect upon, cytoplasmic structures. If the interpretations be correct this would be an example of gene-controlled nucleo-cytoplasmic exchange (41).

MALPIGHIAN TUBULES

The micromorphology of the Malpighian tubule (8) of the grasshopper is remarkably similar to that of the proximal convoluted tubule of the vertebrate. In cross section the Malpighian tubule is seen to consist of four to six epithelial cells, bordered basally by a basement membrane and apically by microvilli. The cells possess distinct cytoplasmic zones, the morphology of which can be correlated directly with the specialized function of the cells.

The basal zone is characterized by labyrinthine infoldings of the plasma membrane. The compartments so formed range from 38 to 160 μ in width and contain rows of mitochondria and vesicles of the endoplasmic reticulum. The intermediate zone of the cell contains the nucleus and few, scattered, round to ovoid mitochondria.

The apical zone is the most striking. The plasma membrane is modified into tightly packed, long (3 to 4 μ) microvilli averaging 1 μ in width. The microvilli contain elongated mitochondria, often expanded at the tips, thus deforming the periphery of the villi into large bulbs. Presumably the bulbous tip secondarily may be pinched off and the membrane-enclosed mitochondria extruded from the brush border. Such a transport mechanism, involving mitochondrial migration and elimination, resembles the phenolic oxidase-producing, Type 2 colleterial gland cell (59) and differs from the more common cytopempsotic and pinocytotic mechanisms usual in protein secretion in which membranes of the endoplasmic reticulum and Golgi complex are involved.

The uratic granules, averaging 1.5 μ in diameter, are formed within peripheral vesicles of the endoplasmic reticulum by secretion of concentric layers of urate. In a given region of the cell large numbers of granules are formed at one time. The accumulated granules move to the apex of the cell, are liberated by rupture of the enclosing membrane, and enter the lumen of the tube almost simultaneously. Some variation in form of granule occurs from species to species (11).

A unique excretory product of the Malpighian tubule is seen in leafhoppers. Designated "brochosomes," they were originally found as netted, hol-

low spheroids, 240 to 600 μ in diameter, in association with the wings and integument. It was believed that they could represent epidermal-cell secretions, reaching the surface by way of the pore canals during integument formation (82 to 85). Their presence on the wings raised the question of the brochosomes being excretory products since leafhoppers smear excreta over the wings. Malpighian tubules of four species of leafhoppers, examined in dried and thin-sectioned preparations (19) indeed showed the presence of brochosomes. The four Malpighian tubules of the leafhopper have large cells in the posterior region and narrow cells in the anterior region where the tubules attach to the filter chamber. The anterior cells possess microvilli on the luminal border and a labyrinthine structure on the basal border similar to the tubules of the grasshopper. The cells of the posterior region, however, possess a scarce cytoplasmic matrix widely partitioned into threads by numerous, membrane-limited vacuoles containing brochosomes in various formative stages. The brochosome reaches the lumen apparently through the rupture of the vacuolar membrane when the latter becomes continuous with the luminal plasma membrane. The brochosome itself has the form of a dodecahedron with a projection from each corner, the body of the structure being surrounded by a membrane. The composition and function of the brochosome are still to be determined.

LIGHT ORGAN

The cells of the reflector layer of the light organ are characterized by urate granules and long mitochondria. The former are of low electron density, finely filamentous, and contained within vacuoles presumably formed by endoplasmic reticulum. The mitochondria are numerous and possess well-formed cristae.

The photogenic layer of the light organ possesses tracheal-end cells and photogenic cells. The spherical to ovoid tracheal-end cell possesses little or no basement membrane. Its nucleus is eccentric, and its cytoplasmic matrix is granular. The tracheal twig, centrally located, is surrounded by a membrane which takes the form of radiating microvilli within which are long mitochondria. The tracheoles in the distal part of the cell possess taenidia and are enclosed within membranes continuous with those of the twig. The tracheolar intima also is surrounded by short microvilli. The mitochondria are confined principally to the body of the end cell, few being in the tracheolar region. The tracheoles do not penetrate the photogenic cells.

The photogenic cells are characterized by dense, spindle-shaped photogenic granules possessing longitudinally oriented lamellae. The granules are located preferentially in the inner cytoplasm of the cell, the cortical cytoplasm containing numerous small mitochondria but no photogenic granules. The cytoplasmic area in which the granules occur contains few mitochondria, small vesicular elements of the endoplasmic reticulum, and multivesicular bodies.

The tracheal-end cell is presumably a modified epithelial cell, the microvilli of which would provide a large surface area for gaseous exchange. The

highly localized distribution of mitochondrial aggregates in both end cell and photogenic cell is presumably related to the energetic mechanisms involved in the luciferase-luciferin reaction (4, 93).

REPRODUCTIVE SYSTEM

Colleterial Gland.—The colleterial glands are accessory glands producing the resilient material of the egg capsule. The left gland produces structural protein, hardening glucoside, and phenolic oxidase. There are four types of cells in the left gland.

Type 4 cells are found at the innermost ends of the tubules and are the most numerous. The single nucleus, at the basal pole of the cell, contains nucleoli, and dense, peripheral, particulate deposits, and is bounded by the commonly described membranes. The ergastoplasmic membranes are massed at the basal pole, more scattered in the apical cytoplasm. RNA (ribonucleic acid) granules occur free in the cytoplasm and also attached to the cytoplasmic side of the reticular membranes. The Golgi complex is mainly vesicular. The mitochondria are closely associated with the endoplasmic reticulum. The "end apparatus" of the cell consists of long, radially arranged, tubular extensions of the cell membrane. It appears open at its tips, suggesting that the secretion droplets pass down the processes and emerge through temporary gaps in the plasma membrane, passing to the main lumen through a chitinous funnel formed by chitinogenic cells lining the apical border of the gland.

Type 2 cells, in the posterior branches of the gland, are taller, have a dense cytoplasm, and possess a more elaborate end apparatus. The latter possesses closely-packed, coarse cell processes beyond which a feltwork of filamentous material separates the secretion from the microvilli. The cytoplasm contains numerous, elongate mitochondria and little ergastoplasm. The mitochondria possibly enter into the formation of the phenolic oxidase as evidenced by their appearance in the processes of the end apparatus. Type 3 cells appear to represent a transition between Types 2 and 4.

The chitinogenic cells are apparently responsible for the secretion of the chitinous intima of the lumen and also for the basement membrane surrounding each tubule. These cells contain numerous mitochondria but little endoplasmic reticulum, suggesting that ribonucleic acid is not required for polysaccharide synthesis.

The structural protein secretion appears as droplets averaging 70 μ in diameter. Calcium-oxalate crystals may appear in the lumen but not in the cells. Hence, their precursors must originate in the cells and the crystals must be formed outside the cell membrane (59).

Oocyte.—Nucleo-cytoplasmic exchange has been studied in *Rhodnius* nurse cells (1). However, fine structural studies of insectan oocytes are lacking. Thus, it is perhaps relevant to describe those of spiders. Oocytes of four spider families, Lycosidae, Thomisidae, Gonyleptidae, and Sicaridae, have been investigated with the electron microscope (77). Members of the first two families have vitelline bodies in the oocytes, the latter two do not.

The vitelline body, close to the oocyte nucleus, consists of a lamellar cortex and a central core. The cortex comprises concentric layers of ergastoplasmic cisternae, long, vesicular mitochondria with few cristae, and small groups of Golgi elements. The central core contains free and clumped vesicles, and multivesicular and capsulated bodies. The latter consist of a capsular layer and contained "geminated masses," which are twin, granular and filamentous, round to ovoid masses, 0.5μ in diameter, and enveloped by a double membrane. Membrane continuities suggest that the inner masses may develop from infoldings of the capsular membrane. The capsular layer is of low electron density and surrounded by a single membrane, which at times may show complicated folds. The vitelline body is unrelated to yolk formation, yolk granules arising in the cytoplasm independently of these organelles. Both similarities and differences exist between the vitelline body and the idiosome of the male germ cell, but their relationship can only be clarified by further studies.

Oocytes without vitelline bodies are characterized by multivesicular bodies of complex structure. Those cytoplasmic components normally found in vitelline bodies may be present but are scattered throughout the cell.

Spermatogenesis.—The final components of the mature sperm of the insect arise by modification of ubiquitous intracellular organelles. The axial filament (10, 14, 81, 91, 92) of the spermatozoa is derived from the centriole. It has the usual ciliary structure of two central and nine peripheral hollow filaments surrounded by a membrane.

The acrosome (9, 14) is formed initially by the Golgi complex. An acroblast (pro-acrosome) in the secondary spermatocyte originally comprises the dictyosomal and vesicular components of the Golgi apparatus. Secondly, the dictyosome enlarges and forms an external, horseshoe-shaped, lamellated component, and the microvesicles aggregate within the enclosure to form the vesicular interior of the acroblast. The terminal macrovesicles of the complex seem to disappear during this development, although they perhaps participate in the formation of the dense acrosome which appears in the open end of the horseshoe arrangement. The acrosome migrates to the front end of the nucleus and becomes electron dense. The remainder of the acroblast is retained in the residual cytoplasm.

The nebenkern (10, 14, 21, 91, 92) arises from mitochondrial fusion. In the primary spermatocyte the mitochondria are randomly distributed. In the early spermatid they assume a juxtanuclear position. The mitochondria elongate and agglutinate, forming a body approximately $5 \times 3 \mu$, in which the outer mitochondria are enlarged and the inner ones decreased in size. The peripheral mitochondria lose most of their cristae until only two hemispherical sacs are left of the original elongating outer pair. The core of the nebenkern is dense, the density being representative of the membranes of the squeezed, small, inner mitochondria. Until this stage, the membranes of the individual mitochondria are visible. With elongation of the spermatid the two nebenkernal hemispheres thin out and grow distally along with the tail filament. During its growth distad, the individual nebenkern "spins out,"

forming a thin filament along which mitochondrial elements are visible. These latter disappear before the final spermatozoan structure is attained, leaving only the two filaments alongside the axial filament in the spermatozoan tail. At least in some insects (91), the matrix of the elongating nebenkern possesses a dense substance, which in late stages occupies practically the entire structure. In the juxtanuclear region of the spermatid of *Drosophila* and *Gelastorhinus* (91), concentric laminated structures designated as chromidial bodies are seen among the mitochondria. The chromidial bodies migrate with the elongating nebenkern, but are not found in the mature spermatozoan tail.

The nuclear changes during maturation (14, 18, 39, 42, 92) involve association of the filaments (chromosomal microfibrils) into a compact body. In the early period of spermatid elongation, the nuclear contents are fibrous; during the middle period longitudinal lamellae are formed by lateral association of filaments; and during the late maturation period the lamellae coalesce into an electron-dense crystalline body. The early filaments average 10 to 15 μ in diameter; the later parallelly oriented filaments average 4 to 7 μ . The finer filaments become arranged in pairs in an anastomosing network, which upon stretching forms a honeycomb pattern. The finer filaments seem to increase in number at the expense of the larger, their initial appearance being correlated with the disappearance of nonbasic proteins (18).

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NEUROSECRETION IN INSECTS¹

BY WILLIAM G. VAN DER KLOOT²

*Department of Pharmacology, New York University
College of Medicine, New York, New York*

INTRODUCTION

A doctrine common not long ago had it that animals are co-ordinated by two virtually independent systems: nervous and endocrine. The discovery and exploration of neurosecretory cells has made this doctrine quite out of date. The neurosecretory cell can stand as a link between nervous and endocrine systems. While it is a neuron in form and probably in function, it may serve as a source of hormones circulating through the tissues of the body, having widespread effects far from the point of origin.

The neurosecretory cell plays a central part in the life of the insect, and the insect has played a central part in our knowledge of neurosecretion. The critical transplantation experiments, which proved the endocrine activity of certain nerve cells, were first performed on insects, and experimental difficulties prohibit the repetition of this experiment in many other animals.

At the outset it should be remembered that neurosecretion in insects has been frequently and ably reviewed and has played a prominent part in two international symposia [Gabe (43, 45); Hinton (68); Possompès (116, 117); B. Scharrer (127); E. & B. Scharrer (129); Wigglesworth (149, 152)]. The available space does not allow a detailed discussion of the comparative anatomy; consequently this paper will emphasize current problems in the physiology of neurosecretory cells.

The definition of the Scharrers—"neurosecretory cells are nerve cells which show cytological evidence of secretion"—furnishes the basis for our discussion. It is most important to keep in mind the cytological orientation of the definition. Certain neurosecretory cells, it is true, release hormones, but there is no reason to assume that this is true of all. For example, Johansson (75) and Schmidt (130) found secretory inclusions in cells which are probably motor neurons. Knowles & Carlisle (82) tried to avoid ambiguity by defining neurosecretory cells as "neurons showing cytological signs of secretion while not innervating a muscle or a gland." In practice it is difficult to check this definition, and it is difficult to decide whether a neurosecretory cell is "innervating" a gland (do the neurosecretory cells of the brain innervate the secretory cells of the *corpora cardiaca*?). Confusion can be avoided by recognizing that a neurosecretory cell is not necessarily an endocrine source. Perhaps it would be best to use a new term and call neurosecretory cells which release hormones "neuro-endocrine" cells.

¹ The survey of the literature pertaining to this review was concluded in April, 1959.

² Assisted by a grant from the Institute of Neurological Diseases and Blindness, Public Health Service.

DISTRIBUTION AND MORPHOLOGY

Insect neurons showing cytological indications of secretory activity were discovered by Weyer (145) in the brain and other ganglia of the honey bee. As interest increased, notable advances were made in the methods for demonstrating neurosecretory cells, particularly the use of chrome-hematoxylin-phloxin and of aldehyde fuchsin, which is shown in Figure 1.A [Scharrer (124); Stutinsky (135); Gabe (42)]. Both methods reveal secretory granules in the perikarya of certain neurons and also often stain secretory granules migrating down the axons, so that the course of the axons can be traced.

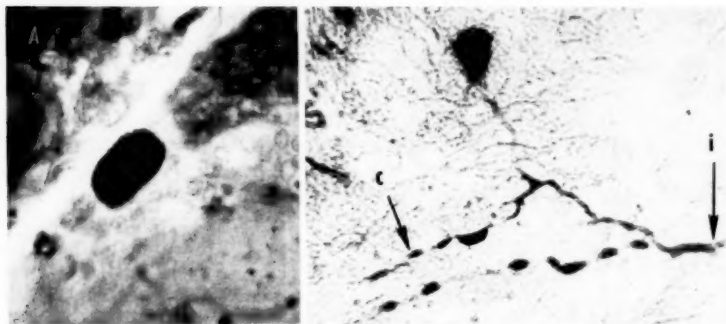


FIG. 1. A. A medial neurosecretory cell in the brain of a diapausing pupa of the cecropia moth. Aldehyde fuchsin B. A silver-stained (114) brain neuron from the cecropia moth. The axon divides and root c runs to the nervus corpus cardiacum of the contralateral hemisphere; root i runs to the same nerve in the ipsilateral hemisphere. This cell is believed to be a neurosecretory cell because of the granules swelling the axons, the course followed by the roots, and the location of the cell body.

The brain.—Weyer immediately saw the similarity between the neurosecretory cells of the insect brain and the X-organ of the Crustacea. The validity of the comparison has become even more impressive with further knowledge. In the Apterygota the neurosecretory cells are separated off in a capsule of connective tissue on the dorsal surface of the brain [Hanström (54, 55), Gabe (44)]. The axons run through the brain and can be traced beyond to the corpora cardiaca. These neurosecretory cells are, judging from their position, probably homologous to the lateral frontal organs of the lower Crustacea, the structures which have evolved into the X-organ. In both insects and crustaceans, the further evolution has seen the incorporation of the neurosecretory cells into the brain.

In the Pterygota the medial neurosecretory cells are located on the dorsal surface of each hemisphere of the protocerebrum, closely adjacent to the midline, that is, in the region known as the pars intercerebralis. The axons run downward, and many or all then cross over into the opposite hemisphere.

They run backward in the brain and exit as a small nerve, the nervus corpus cardiacum I. The nerve runs to the corpus cardiacum, but at least some of the axons continue through the corpus cardiacum and enter the corpus allatum [Arvy, Bounhiol & Gabe (3); Bounhiol, Gabe & Arvy (7, 8)]. The axon endings in the cardiacum are close to the aorta and sometimes actually enter the wall of the aorta [Benwitz (4)].

The corpus cardiacum is frequently connected to the brain by a second nerve containing neurosecretory axons, the nervus corpus cardiacum II. The cell bodies for these axons vary in location according to the order of insects studied. In some Diptera and Hymenoptera the cell bodies lie in the medial group [Brandenburg (9); Cazal (13); L'Helias (91); Thompson (139)]. In other insects, the cell bodies lie as a separate lateral group, often found just medial to the corpus pedunculatum in the pars intercerebralis. In still other insects the lateral group lies between the corpora pedunculata and the optic lobes, outside of the par intercerebralis. The axons of the lateral cells run to the nervus corpus cardiacum II without decussating. The lateral cell group is found in the Lepidoptera [Williams (154, 155)], Dermaptera [Lhoste (95, 96)], Homoptera, Ephemeroptera, and Brachycera [Cazal (13)], Hemiptera [Nayar (102, 105)], and some Diptera [Köpf (87, 88)].

The significance of the lateral cells is enhanced because they are often seen only in the later stages of postembryonic life, which suggests that they have a special role to play [Arvy, Bounhiol & Gabe (3); Bounhiol, Gabe & Arvy (7, 8); Formigoni (33); Kobayashi (84); Lhoste (95)].

Outside the protocerebrum, one or two neurosecretory cells were found by Dupont-Raabe (20 to 23, 27) in the tritocerebrum of *Carausius morosus* Brunner.³ The axons run to the corpus cardiacum as nervus corpus cardiacum III.

Other groups of cells in the brain are also neurosecretory; in fact, Frazer (34) describes six groups in each hemisphere of the brain of *Lucilia caesar* Linnaeus.

Other ganglia.—Neurosecretory cells are also commonly found in other ganglia of the insect central nervous system. In some insects the subesophageal ganglion shows a more vigorous picture of secretory activity than does the brain itself [Scharrer (123); Vazquez & Villa-señor (144)]. Moreover, the number of neurosecretory cells in the ganglia of the ventral nerve cord may far outweigh the number in the ganglia of the head. In *Bombyx mori* (Linnaeus), for example, Kobayashi (84) finds about 30 neurosecretory cells in the brain, 80 in the subesophageal ganglion, and 1100 in the thoracic and abdominal ganglia. Neurosecretory cells are also seen in the frontal ganglion and in the corpora cardiaca.

Types of cells.—Selective staining clearly shows that distinctions can be made among the neurosecretory cells of the brain. Nayar (102, 105) distin-

³ For the benefit of physiologists, it is worth noting that this insect was until relatively recently known as *Dixippus morosus* (Brunner). My feelings on name changing are perfectly expressed by Williams (160).

guished two varieties: A-cells (dark blue with chrome-hematoxylin, purple with aldehyde fuchsin) and B-cells (red with phloxin; green with aldehyde fuchsin). This classification seems most useful and was followed by Johansson (75), Kobayashi (84) and Köpf (87, 88). Johansson (75) also described additional cell types. Some authors believe that the categories represent one type of cell seen in two phases of a secretory cycle [Brandenburg (9); de Lerma (17), Herlant-Meewis & Paquet (63), Nayar (102); Thompsen (136)]. This interpretation is supported by the discovery of red- and blue-staining granules within the same cell [Lhoste (95, 96); Thompsen (139)]. And Scharrer (126) experimentally transformed cells from purple-staining to green. On the other hand, the lateral neurosecretory cells of *B. mori* are always of the B-type, which shows that differences in the stain taken up may reflect persistent distinctions between cell types [Kobayashi (84)].

Histochemistry and ultrastructure.—Only a few histochemical observations have been made on neurosecretory cells. Sloper (132, 133) showed that the secretory granules in *Leucophaea maderae* (Fabricius) stain with a method for protein-bound cystine and cysteine, just like the neurosecretory substance of vertebrates. Nayar (103) demonstrated that the neurosecretory cells of *Iphita limbata* Stål contain acid phosphatase and cholinesterase, but found no succinic dehydrogenase or alkaline phosphatase. Kobayashi (84) stained the medial neurosecretory cells of the silkworm for lipids, carbohydrates, and proteins.

Similarly, only a promising start has been made in exploring the ultrastructure of neurosecretory cells. Meyer & Pflugfelder (98) looked at the corpora cardiaca of *C. morosus* with the electron microscope. The neurosecretory material is in the form of spherical granules, about 1000 Å in diameter, bounded by a distinct membrane.

NEUROSECRETION AND MOLTING

Kopec (86) showed that the brain is necessary for the development of *Porthetria dispar* (Linnaeus); when larva were deprived of the brain before a critical moment in the last instar, they never pupated. Moreover, the brain was acting as an endocrine organ, because reimplantation of the brain allowed molting and metamorphosis to proceed. This important result was confirmed by Caspari & Plagge (12), Bounhiol (5), and Kuhn & Piepho (90).

Further advance has been made by Wigglesworth (146), who had shown that an important endocrine for the molting of *Rhodnius prolixus* Stål is in the head. Hanström (53) then described the medial neurosecretory cells of *R. prolixus*, and Wigglesworth (147), following this lead, proved that the molt was triggered by a hormone produced in the pars intercerebralis of the brain.

The neurosecretory cells are now known to be essential for molting in a large number of insects; recent demonstrations are by Church (16), Fukaya (35), Ichikawa (69), Ichikawa & Yashika (70), Kobayashi (83), and Nayar (104). Nevertheless, the brain is not always needed for molting. Johansson

(75, 76) removed the medial neurosecretory cells of *Oncopeltus fasciatus* (Dallas) without interfering with the molt. Perhaps in this insect the corpora allata act in the place of the brain (see below). In spite of this exception, it seems safe to say that the medial neurosecretory cells of many insects are neuro-endocrine cells releasing a hormone which triggers the molt.

The sequence of events in the cells seems reasonably clear. The hormone appears to be synthesized in the perikaryon and is then transported within secretory granules moving down the axon. The movement down the axon was demonstrated by Scharrer (125) and by Thompson (137), who performed the unmatched feat of ligaturing the nervus corpus cardiacum I of *Calliphora erythrocephala* (Meigen) and showed that the ligatured axons filled up with secretory material from the brain. The transported material may then be stored at the axon endings in the corpora cardiaca and corpora allata. The release into the circulation probably comes at these points, close to the principal circulatory stream of the insect head. Therefore, the corpora cardiaca is an excellent example of a neurohemal organ, in the sense of Carlisle & Knowles (11)—an organ where neurosecretory material is released into the blood. In opposition to this interpretation, Kobayashi (84) presents photographs indicating that some secretory granules are released from the cell body directly into the brain. However, the insect central nervous system is surrounded by a dense sheath, impermeable to many small molecules; a specialized structure for the release of the hormone seems almost a necessity.

Corpora cardiaca and molting.—It is important to recognize that the corpora cardiaca is not always needed for hormone release. Transplanted brains which are deprived of the corpora cardiaca continue hormone release, and Piepho (113) found that removing the corpora cardiaca does not hinder the pupal molt of *Galleria mellonella* (Linnaeus).

The corpora cardiaca are also unnecessary in the cecropia moth [*Hyalophora cecropia* (Linnaeus)]. This was for a time somewhat mysterious because Williams (155) showed that both medial and lateral neurosecretory cells are required for hormone production. And it is also important that the two groups of cells be in one piece of brain. Williams suggested that the medial and lateral cells collaborate to produce a single developmental factor. It was difficult to see where this collaboration might take place when the corpora cardiaca are removed. The puzzle was resolved by Stumm-Zollinger (134), who studied Williams' preparations and showed that the cut ends of the neurosecretory cells regenerate a structure outside the brain in which the axons of the medial and lateral neurosecretory cells intermingle. In the cecropia moth at least some of the axons from the medial neurosecretory cells appear to divide and run to both right and left corpora cardiaca (Figure 1B). In further experiments the neurosecretory cells are burned from the *in situ* cecropia brain by using a microcautery [Van der Kloot (143)]. The hormone was produced as long as one medial and one lateral group remained intact. So all experiments favor the important idea that the medial and lateral

neurosecretory cells produce distinct substances which are joined at the axon endings to form a single hormone.

There is no indication of how widespread the interaction of medial and lateral cells may be. The mechanism is obviously different in the *Cyclorhapha*, where Possompès (118, 119) showed that molting is prevented by transecting the *nervus corpus cardiacum*.

The action of the brain.—At just the time when it was becoming clear that the molt of many insects is triggered by neurosecretory cells in the brain, Fukuda (37) discovered that the prothoracic glands of *B. mori* secrete a molting hormone. It then seemed that molting must be controlled by two completely independent organs. Williams (154 to 157) put the facts together and showed that the brain releases a tropic hormone which stimulates the prothoracic glands. It is the prothoracic gland hormone which acts on the tissues of the body to produce the changes characterizing the molt. This unifying idea was extended to the Hemiptera [Wigglesworth (148)], Orthoptera [Pflugfelder (112)], Odonata [Deroux-Stralla (18)], Mecoptera [Rahm (120)], and Diptera [Possompès (115, 116)]. It is technically difficult to remove both prothoracic glands and show that molting is blocked; this has been done only once [Joly, Joly & Halbwachs (76)]. The stimulation of the prothoracic gland by the brain is important even in embryonic development [Jones (77, 78)].

On the other side, Chadwick (14, 15) proved that *Periplaneta americana* (Linnaeus) can molt after the removal of the prothoracic glands. And Wigglesworth (150, 151) showed that the hematocytes are in some way involved in activating the prothoracic glands of *R. prolixus*. In spite of this exception and complication, the triggering of the prothoracic gland by the brain seems clearly established for many insect orders.

Secretory cycles.—Along with the evidence for the triggering of the molt by a hormone released from the brain, it is not surprising to find histological signs of an appropriate secretory cycle in the neuro-endocrine cells. A clear secretory cycle, correlated with molting, was found by Rahm (121, 122) in *Anagasta kuhniella* (Zeller), and in other species by other investigators; but in certain species a clear correlation is not seen [Herlant-Meewis & Paquet (63)]. The species studied may differ because of a real variability in the part played by the neuro-endocrine cells; or they may differ in the extent to which cytological changes provide a measure of hormone release.

Chemistry of the hormone.—The neuro-endocrine cells of the insect are commonly assumed to follow the pattern established in the vertebrate posterior pituitary; releasing a hormone of low molecular weight. The secretory granules are believed to be a carrier for the hormone. L'Helias (93, 94) suggested that the brain hormone is a derivative of pteroyl glutamic acid. The evidence is the presence of pterins in the brain and the reported ability of folic and folinic acids to shorten the intermolt period in *C. morosus*. However, Williams [(155) quoted in (79)] showed that folic acid was without effect in the cecropia silkworm and that folic acid antagonists did not hinder development.

More recently, Kobayashi & Krimura (85) obtained 2 mg. of ether soluble extract from 8500 silkworm brains; the injection of 0.1 mg. of this material into a brainless *dauer* pupa led to adult development. Clearly this extract mimics the brain hormone by activating the prothoracic glands. This significant result may be somewhat surprising because the brain factor involved at least superficially resembles the extracts with corpora allata hormone activity prepared by Williams (158), which is also ether soluble. The resemblance is even more impressive. Williams (160) has shown that the action of the brain is mimicked by injection of extracts with corpora allata hormone activity. Also the prothoracic glands can be triggered by implantation of a number of active corpora allata [Ichikawa & Nishiitsutsujo-Uwo (71); Williams (160)]. It is not yet certain whether the corpora allata hormone is triggering the prothoracic gland or whether the extracts include the hormone from the brain. The implanted corpora allata might act by releasing stored neurosecretory material, which originated in the brain. The neurosecretory material is tenaciously stored in the corpora allata [Bounhiol (6)]. The other possibility is even more attractive. The two hormones may be close in structure and action; the corpora allata hormone itself may activate the prothoracic gland. If this interpretation is correct, in some insects the corpora allata may normally trigger the larval molts. This would explain how the larval molt of some insects continues after the brain is removed.

The control of hormone release.—An outstanding feature of insect life is the periodic molt, which interrupts the life cycle at definite time intervals or according to the course of external events. In only a few instances is there any indication of the mechanism which regulates periodic hormone release.

Wigglesworth (146) showed that the molt of *Rhodnius prolixus* is invariably triggered by the ingestion of a blood meal. The nutritional content of the meal is unimportant; the stimulus comes from the swelling of the abdomen. Hormone release begins within minutes after feeding and must continue for three days if a molt is to follow [Wigglesworth (153)]. Molting is prevented when the nervous pathway between the abdomen and the brain is interrupted after feeding. Wigglesworth concluded that afferent impulses, set up by stretching the abdomen, are conveyed through the ventral nerve cord to the neurosecretory cells of the brain. Recently Van der Kloot (143)—using electrophysiological methods—found receptors in the abdomen of *R. prolixus* which are activated when the abdomen is stretched. It is also possible to record from the nervus corpus cardiacum. In an unfed animal impulses are rarely recorded from the nerve. After feeding, some of the neurons begin to conduct about three impulses per second. In an unfed bug, impulses can be set up by stretching the abdomen. This result supports Wigglesworth's interpretation and adds the information that axons from the brain conduct impulses when the neurosecretory cells are activated. It is not certain that the impulses are in neurosecretory axons because Stumm-Zollinger (134) showed that there are other axons in the nervus corpus cardiacum I of the cecropia moth. Yet it would be reasonable to find that hormones are released

at the endings of neuro-endocrine cells under the same conditions in which ordinary neurons release neurohumors, namely with the arrival of a nerve impulse at the axon ending. It seems unlikely that animals evolved neurons which release hormones, while at the same time the ability to control the release of chemical was discarded.

Monro (99) argues that the brain of *Phalaenoides glycine* Lewis is stimulated by a chemical to release the hormone. The evidence is based on the assumption that an isolated brain cannot release hormone in a larva during the period of obligatory feeding. I do not know the evidence for this assumption.

Diapause.—A second instance in which there is some information about the regulation of hormone release is the pupal diapause of certain saturniids. Williams (154, 155, 156) showed that diapause results from the failure of endocrine activity by the brain. Shortly before the pupal molt, the brain stops releasing hormone and secretion is resumed, weeks or months later, just before the visible onset of adult development. The return of the brain to secretory ability is promoted by low temperatures [Williams (159)]. Van der Kloot (141, 142) reported that the loss of endocrine activity is paralleled by the disappearance of spontaneous electrical activity and of cholinesterase from the brain. Low temperatures promote the accumulation of a cholinergic substance in the brain, a substance which may eventually trigger the synthesis of cholinesterase. It was suggested that the neurons of the entire brain are inactivated to insure the inaction of the neurosecretory cells. The resumption of hormone release, just before adult development, is accompanied by the return of cholinesterase and electrical activity to the brain. Apparently, the same mechanism governs the inaction of the brain during the pupal diapause of *Anthereae pernyi* Guérin [Koshtoyants (89)]. It is noteworthy that Monro (100) demonstrated that inhibitors of cholinesterase can block the release of the brain hormone.

The brain also controls the larval diapause of *Cephus cinctus* Norton [Church (16)] and the nymphal diapause of *Gryllus campestris* (Linnaeus) [Sellier (131)].

On the other hand, the diapause of *Sialis lutaria* (Linnaeus) results from the inaction of the prothoracic gland [Rahm (120)]. And Fukaya & Mitsuhashi (36) showed that the diapause of *Chilo suppressalis* (Walker) can be broken by removing the corpora allata and corpora cardiaca. Here a factor from the corpora allata seems to prevent the release of the brain hormone. In this regard, Highnam (66, 67) has histological evidence for a role of the corpora allata during the pupal diapause in *Mimas tiliae* (Linnaeus), but removal of the corpora allata does not end diapause. Clearly the brain is not invariably the endocrine organ enforcing diapause.

OTHER ENDOCRINE ROLES OF THE BRAIN

Color changes.—The importance of the neurosecretory cells of the brain in controlling growth must not obscure the clear evidence for other functions

of hormones released by neurosecretory cells in the brain. Dupont-Raabe (21 to 28) has shown that an organ in the head exerts an endocrine control over the diurnal color changes of *C. morosus*. Ablation experiments failed to show a role for the corpora cardiaca or corpora allata. The critical organ is the brain; brain removal leads to a permanently light animal. Brainless animals darken after the injection of extracts of the corpus cardiacum or of the tritocerebrum; extracts of the pars intercerebralis are without effect. The tritocerebrum contains neurosecretory cells (see above) and the axons run to the corpora cardiaca. However, the chemical factor in the corpora cardiaca is distinct from the chemical of the tritocerebrum. The substance from the corpora cardiaca is a peptide similar to the color-changing hormone of the shrimp [Knowles, Carlisle & Dupont-Raabe (81)]. The tritocerebral factor of Dupont-Raabe may be the same as the "chromatotrope" of L'Helias (92, 93, 94).

Activity rhythms.—Two recent papers suggest that hormones from the neurosecretory cells of the brain play a part in the control of activity rhythms. Klug (80) studied *Carabus nemoralis* Mueller which has both diurnal and yearly activity cycles. The rhythms appear to be correlated with changes in the volume of the nuclei of the cells of the corpora allata and with changes in the number of neurosecretory cells in the brain containing secretory granules. For example, at noon he finds 15 cells in the brain loaded with neurosecretory material, while at 4 A.M. all are empty.

The second piece of evidence comes from Ozbas & Hodgson (111), who showed that extracts of the corpora cardiaca of *Periplaneta americana* decrease the spontaneous electrical activity of isolated ventral nerve cords, while extracts of the brain, corpora allata, or abdominal ganglia are ineffective. An injection of the corpora cardiaca extract into whole roaches caused inco-ordination and stereotyped locomotor behavior; the symptoms developed to a maximum after several hours and persist for 24 to 96 hr. Apparently the active material comes from the neurosecretory cells of the brain—even though brain extracts are inactive—because the extracts were less active when made from corpora cardiaca whose connections to the brain were severed several weeks earlier.

Water metabolism.—Considering the forceful analogy between the insect system and the vertebrate posterior pituitary, surprisingly little has been done on the possibility that the neurosecretory cells of the insect brain affect water metabolism. Nuñez (110) studied the regulation of water content in *Anisotarsus cupripennis* Germar. The normal beetles take water in through the body surface and the excess is excreted by the Malpighian tubules. The rate of excretion is regulated so that the water content of the body is constant. The regulation of excretion is disrupted if the circulation to the head is cut off by a ligature, if the connectives between the brain and the subesophageal ganglion are cut, or if the dorsal part of the brain is removed. Extracts of the brain and corpora cardiaca, when injected into ligatured larvae, promote water retention for about half an hour. Subesophageal gan-

gion extracts are ineffective. Nuñez also showed that water regulation is disrupted when abdominal ganglia are removed; the closer the ganglion removed lies to the thorax, the greater the extent of the disruption. Nuñez believes that the abdominal ganglia contain sense organs which promote the release of the brain factor.

Altmann (1, 2) showed that the injection of extracts of the corpora cardiaca into the honey bee leads to a decreased water excretion and an increased blood viscosity. Corpora allata extracts had the opposite effects (extracts of Malpighian tubules and of fat body did nothing). Both the active extracts resisted heating. The corpora cardiaca extract was mimicked by epinephrine, and on this basis Altmann suggests that the active principle is an orthodiphenol, perhaps related to the substance of Cameron (10). If this proves true, neurosecretion may not be involved in these discoveries.

Nayar (106) studied water metabolism in *Iphita limbata*. Forcing the insect to drink distilled water leads to the production of a copious urine and at the same time the medial neurosecretory cells are emptied of secretory granules. When forced to drink salt water, the insects excrete little urine, the neurosecretory cells are choked with granules, and little secretory material is seen near the aorta.

It seems quite certain that the medial neurosecretory cells are involved in the control of water retention.

Reproduction.—Removal of the medial neurosecretory cells from adult female *Calliphora erythrocephala* stops egg development at the stage when yoke deposition normally begins [Thompson (136)]. The removal of the lateral neurosecretory cells has less effect. The corpora cardiaca are not needed for normal egg development, but implanting "activated" corpora cardiaca into brainless animals promotes egg maturation. Thompson (136) suggested that the medial neurosecretory cells influence protein metabolism because a similar failure in egg development was seen in flies fed on sugar and never given meat.

A seeming experimental inconvenience in the original experiments has now been turned to good account. Egg development is also affected merely by severing the nervus oesophagei, which runs from the corpus cardiacum to the anterior part of the gut. Thompson (138) has now shown that the n. oesophagei transport neurosecretory granules from the brain to the gut. Interrupting the transport results in a greatly reduced proteinase activity in the gut. So neurosecretion may be needed for the proper digestion of proteins, and restricted amino acid intake may be responsible for slowing egg growth.

Nayar (107, 108) finds that transplanting medial neurosecretory cells into a gravid female of *I. limbata* almost immediately stimulates oviposition—even when the eggs are immature. In the normal female oviposition coincides with the depletion of material in the neurosecretory cells. Nayar's experiments also show that the release of neurosecretory material and ovi-

position are promoted by a heat-stable material present in extracts of the ovaries.

In agreement with these results, Noirot (109) detects secretory activity in a group of neurosecretory cells, at the base of the optic ganglion in adult and in supplementary reproductives of *Kaloterms flavicollis* (Fabricius). Dupont-Raabe (26) sees vigorous secretory activity in the par intercerebralis of *C. morosus* at the time of egg maturation. And Gillett (51) has evidence from ligature experiments for the importance of the brain in the development of eggs in *Aedes aegypti* (Linnaeus).

On the other hand, Johansson (73, 74, 75) finds that the removal of the medial neurosecretory cells from *O. fasciatus* does not prevent egg formation, although fecundity is reduced. In this insect the corpora allata are vital for egg development Johansson (72). In *Rhodnius prolixus* [Wigglesworth (152)], *Carausius morosus* [Dupont-Raabe (19)] and *Hyalophora cecropia* [Williams (157)] eggs develop in the absence of the neurosecretory cells of the brain. At present sweeping generalizations about the endocrine control of egg development cannot be drawn.

SUBESOPHAGEAL GANGLION

Neurosecretory cells were known for years to be in the subesophageal ganglion, but all evidence about the function of these cells is recent and many approaches await further exploration. For example, Scharrer (126) found a pair of neurosecretory cells in castrated females of *Leucophaea maderae* which stain an iridescent green. The existence of these "castration" cells suggest an endocrine link between the gonad and the subesophageal ganglion in this species. Hidaka (64, 65) reported ligature experiments which indicate that the color of the pupal cuticle of *Papilio xuthus* Linnaeus is controlled by a hormone released from the head. Ablation studies indicate that the subesophageal and prothoracic ganglia are the source of this factor.

Egg diapause.—Fukuda (38 to 41) and Hasegawa (60, 61) found that the subesophageal ganglion is an important source of a hormone which is released into the maternal circulation and which causes the newly laid eggs of the silkworm to enter diapause. The critical experiment is the transplantation of a subesophageal ganglion from a larva expected to lay diapausing eggs into one expected to lay normal eggs. Many of the females which had received the implants laid diapausing eggs. Even though implantation of the brain by itself has no effect, the number of moths laying diapausing eggs is increased when the brain-subesophageal ganglion complex is implanted. The effectiveness of the complex depends on the continuity of at least one connective between the brain and the subesophageal ganglion. Univoltine silkworms (expected to lay diapausing eggs) lay normal eggs after both subesophageal connectives are severed. In these animals the brain seems to stimulate hormone release. Bivoltine silkworms (expected to lay normal eggs)

lay at least some normal eggs after the connectives are severed. It is apparent that the brain can either stimulate or inhibit hormone release.

The hormone probably comes from neurosecretory cells; Kubayashi (84) found that animals expected to lay diapausing eggs have more large (above 30μ) neurosecretory cells than do other silkworms. The situation was recently complicated by the evidence of Morohoshi (101), which shows that the corpora allata are also involved in the production of egg diapause.

The egg-diapause hormone is also in the first stages of chemical exploration. Hasegawa (62) has extracted with methanol the brains and subesophageal of 15,000 silkworms. The injection of this extract causes normal females to lay diapausing eggs. The active material is soluble in chloroform but not in ether.

Activity rhythms.—After seeing evidence for the brain as an endocrine source regulating activity, it is disconcerting to find that the subesophageal ganglion is also implicated. However, Harker (56, 57, 58) presented evidence, from parabiosis experiments, that the diurnal burst of activity which *Periplaneta americana* normally shows during the first hours of darkness is controlled by endocrines. Implantation experiments show that the source of the hormone is the subesophageal ganglion. Moreover, the injection of extracts of the subesophageal ganglion is reported to produce a burst of activity by a headless roach, the duration of the activity depends on the time of day when the injection is given [Harker (59)].

An endocrine mechanism seems ruled out for the activity rhythm of *Carausius morosus*; Eidmann (30) showed that the rhythm is lost when the brain is removed and is not restored with the reimplantation of the brain.

NERVOUS CONTROL OF ENDOCRINE SECRETION

The considerable interest in the neurosecretory cell must never obscure the fact that endocrine organs may be stimulated by nerve impulses. To cite an excellent example, Englemann (32) and Englemann with Lüscher (31, 97) showed that the corpora allata of *L. maderae* are controlled by an inhibitory pathway from the protocerebrum and by an excitatory pathway from the subesophageal ganglion. Neither innervation seems to depend on neurosecretion.

EFFECTS OF EXTRACTS OF NERVOUS TISSUE

A considerable literature now exists on the effects of extracts of insect nervous tissue on the movements of chromatophores and muscles [summarized by Gersch (48)]. For example, Enders (29) finds that extracts of the brain of *C. morosus* increase the rate of movement of the oviduct and the Malpighian tubules but slow the movements of the gut. Corpora allata extracts depress the oviduct and stimulate the gut. It is uncertain whether these results bear any relation to neurosecretion. The same uncertainty applies to the substance isolated by Cameron (10) from the corpora cardiaca of *P. americana*. This substance stimulates the isolated heart and hind-gut,

but inhibits the fore-gut. The evidence suggests that this material is an orthodiphenol formed in the cells of the corpora cardiaca.

Extracts of the nervous systems of *P. americana* and of *C. morosus* were partially purified by paper chromatography. When injected into *C. morosus*, the extracts cause a change in color [Gersch & Mothes (46)]. Two factors are known: one ("Hormone C") causes darkening, the second ("Hormone D") causes lightening. The same substances act on the melanophores of larvae of *Corethra* [Gersch (47)]. "Hormone C" expands the melanophores, as do acetyl choline and epinephrine; "Hormone D" and nor-epinephrine contract the melanophores. "Hormone D" is distributed throughout the central nervous system and the corpora cardiaca. "Hormone C" is found in all of these organs and also in the corpora allata [Gersch & Unger (50)]. Dupont-Raabe (24) also found a melanophore-expanding substance in the brain and corpora cardiaca of *Corethra* (see above); and Hadorn & Frizzi (52) found an melanophore-expanding principle in the subesophageal ganglion. "Hormones C and D" also act on the frog and insect hearts [Gersch & Deuse (49); Unger (140)]. The relation of these chemicals to the endocrinology of the insects remains to be determined.

CONCLUSIONS

The concern of this paper has been the physiology of the insect neurosecretory cells which are known to release hormones. These particular cells could more fittingly be called neuro-endocrine cells. The reason for the distinction is that in the insect only a handful of the neurons containing secretory granules have been shown to release hormones. In the silkworm, for example, only one-tenth of the neurosecretory cells are thought to release all the known hormones. Moreover, there is no compelling reason to assume that further work will show that all of the remaining nine-tenths of the neurosecretory cells release a hormone at all. Consequently, it seems best to single out the neurosecretory cells which release hormones as neuro-endocrine cells. The distinction is needed because a neurosecretory cell is defined cytologically, while a neuro-endocrine cell is demonstrated physiologically.

This review includes many facts which could not be gathered into a tidy whole. In part this results from our fragmentary knowledge. But it seems quite probable that further studies in insect endocrinology will add more exceptions and complications than unifying generalizations. The insects began with several endocrine organs as part of their arthropod heritage. Other endocrines, like the corpora allata, were introduced during the evolution of the insects. It is only reasonable to suppose that many specialized uses of the endocrine possibilities have arisen while insects traveled their diverse evolutionary paths. To illustrate this point, consider the part played by the medial neurosecretory cells of the brain in promoting egg development. *Calliphora erythrocephala* requires the medial neurosecretory cell for egg maturation. In *Iphita limbata* the medial neurosecretory cells trigger oviposition, while in many other insects the medial neurosecretory cells are not at all needed for

egg development. This is typical of the variability we must be prepared to face as our knowledge of insect endocrinology increases.

On the other hand, chemical investigation may lead to simplifications. To name a few, active extracts have now been obtained which mimic the prothoracic gland-triggering hormone of the brain, the egg-diapause hormone of the subesophageal ganglion, and the color-changing hormone of the tritocerebrum. When the chemicals are identified and available, we will enter a new exciting phase. The use of chemically distinct substances will tell us how many hormones are actually secreted by the neuro-endocrine cells. For example, there is good evidence for a bewildering variety of functions by secretions from the protocerebrum: (a) triggering the prothoracic glands, (b) stimulating proteinase synthesis in the gut, (c) promoting water retention, (d) stimulating oviposition, (e) perhaps controlling activity. It is impossible to decide whether five distinct hormones are required for these functions or one is enough. The initial steps in the separation of the prothoracic gland-stimulating hormone show the way toward the solution of these difficulties.

Even when these problems are solved, some of the most pressing questions remain. To be effective, an endocrine organ must be regulated so that hormone release meets the needs of the animal. If we assume that neuro-endocrine cells release hormones when a nerve impulse arrives at the axon ending—and there is now some evidence for this idea—then the secretion of hormone may be governed by any of the sense organs acting through the integrative networks of the central nervous system. In only a few cases is there any information about the stimuli controlling hormone release. In *Rhodnius prolixus* the abdominal stretch receptors drive the neuro-endocrine cells of the brain. In *Anisotarsus cupripennis* osmo-receptors in the abdominal ganglia may be involved, while in *I. limbata* a chemical produced by the cells of the ovary serves as the trigger. The study of the control of the neuro-endocrine cells promises to become one of the most vigorous fields in insect endocrinology [see Scharrer (128)].

Much of our thinking about neurosecretory cells in all animals comes from the study of insects. It would be surprising if this were not so in the future also.

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THE PHYSIOLOGY OF EXCRETION IN THE INSECT¹

BY RODERICK CRAIG

University of California, Berkeley, California

Excretion is the process by which an organism maintains its internal environment within physiologic limits by eliminating the excess of metabolites. This process operates in all cells, and the enhancement of this function in cells assembled into an excretory structure, or organ, will not alter too much the cells' basic activities. Passage of material into or out of a cell is through the cell membrane. In an excretory organ composed of a layer of cells, material must pass through the cell wall twice, and through the cytoplasm. This increases the importance of the cell membrane in describing the process. Substances may either diffuse through a cell wall because of a concentration gradient, or they may be actively transported even against a concentration gradient. In a terrestrial animal the transport of material into or out of the body as a whole requires energy, which may be supplied by some other region than the one immediately involved in the transfer of substances. It is also obvious that any substance being transported through a cell is subject to the metabolic activities of the cell, as Ramsay (48) found for valine, so that a simple analysis of material entering versus that leaving must be carefully interpreted. This metabolic aspect of excretory structures is perhaps the complement of the excretory aspect of metabolic structures.

THE PRODUCTS OF EXCRETION

The recent developments in analytical microchemistry, especially the use of paper chromatography, have made possible the study of the chemical composition of minute amounts of body fluids such as blood and urine of insects. The potentially more effective isotopic methods have been little used, probably because of expense and unfamiliarity. These newer methods have made possible the study of the products of the Malpighian tubes as a separate material uncontaminated by food residues from the intestine. In insects where the products of the tubes or of the gut are modified by the hind-gut, the term urine becomes ambiguous. It will be better, then, to specify the region from which the excretory material is taken. It must be remembered that the composition of the excreta depends on the diet and on the physiological condition of the insect. Thus the composition of the honeydew of the aphid *Tuberolachnus salignus* (Gmelin) closely parallels the composition of the host sap as it undergoes seasonal changes [Mittler (36)]. The change in excretory fluid composition is even more startling in blood feeders, for example, *Aedes aegypti* (Linnaeus) which shows a decrease in urinary nitrogen from 11.5 per cent immediately after a blood meal to 1.5 per cent after two weeks on a sugar diet [Terzian, Irreverre & Stahler (59)]. The im-

¹ The survey of the literature pertaining to this review covers the period from 1950 to May of 1959.

TABLE I
AMINO NITROGEN DISTRIBUTION IN HONEYDEWS

Amino Compound	<i>M. pisi</i> *†			<i>M. circumflexus</i> ‡§	<i>P. brevises</i>	5 species of Coccoidea#	<i>B. brassicae</i> **
	4 Instar	Adult	Gravid				
α -Alanine	0.18	0	0	1.25	+	+	+
β -Alanine				0.25			trace
α -Amino- n -butyric acid				0.30			trace
γ -Amino-butyric acid	0.37	0.16	0.10				
Arginine				30.0		+	+
Asparagine				60.0	+		
Aspartic acid				20.0	+	+	
Cysteic acid				0.44		+	
Cystine	trace	0	0		+		trace
Glutamic acid	0.64	0.45	0.38	20.0	+	+	
Glutamine and Arginine	1.97	0.84	0.38	60.0	+	+	
Glycine	0.11	0.07	0.03	0.85	+		+
Histidine				10.0	+		trace
Homoserine	5.56	2.86	0.84				
Leucine and isoleucine	2.37	2.10	0.98	4.54			+
Lysine				6.6			+
Methionine sulfoxide	0.40	0.19	0.10	3.42			+
Phenylalanine	0.97	0.51	0.11	3.33	+		trace
Proline	0.68	0.22	0.22	1.50	+	+	
Serine	0.44	0.26	0.11	7.0	+	+	
Threonine	0.76	0.53	0.35	3.75	+	+	+
Tryptophan				8.8	+		+
Tyrosine	0.37	0.11		10.1			trace
Valine	1.23	1.26	0.45	2.4	+	+	+

* μ gm./liter.

† Auclair (1).

‡ mg./100 m. liter of honeydew solution.

§ Maltais & Auclair (28).

|| Gray (15).

Ewart & Metcalf (11).

** Lamb (23).

portance of the physiological state of the insect is indicated by the change in amino acids excreted by the pea aphid, *Microsiphum pisi* Kaltenbach [= *Acyrtosiphum pisum* (Harris)]. Auclair (1) showed that the total amino acid content of the honeydew changed from 1.83 per cent in the nymph to 1.13 per cent in the adult and 0.45 per cent in the gravid adult.

It is only for the aphids and coccids that we find nearly complete analyses of the excretory products, and even these analyses are often qualitative. The most complete quantitative analyses for amino acids in honeydew are shown in Table I for three stages in the life cycle of the pea aphid, *M. pisi* by Auclair (1) and for the crescent-marked lily aphid, *Myzus circumflexus* (Buck), by Maltais & Auclair (28). The honeydew from the cabbage aphid, *Brevicoryne brassicae* (Linnaeus), as analyzed by Lamb (23), contains 2.35 per cent amino compounds of which glutamine and asparagine comprised 0.85 per cent wet weight. Eighteen amino acids were also present. The total

amino acid content of honeydew closely paralleled that of the plant sap, and the qualitative analysis was similar. Mittler (36) found fewer amino acids in the honeydew of *T. salignus*, and they were the same ones present in the plant sap. The honeydews produced by five species of coccids—the cottony-cushion scale, *Icerya purchasi* Maskell, the soft scale, *Coccus hesperidum* Linnaeus, the citrus mealybug, *Planococcus citri* (Risso), the citricola scale, *Coccus pseudomagnoliarum* (Kuwana), and the black scale, *Saissetia oleae* (Bernard)—have been studied by Ewart & Metcalf (11). The amino-containing compounds found in the honeydews (arginine, aspartic acid, glutamic acid, asparagine, glutamine, serine, threonine, alanine, proline, and valine) were the same for each species even though the host plants were different. Gray (15) detected 19 amino-containing compounds in the honeydew of the pineapple mealybug, *Pseudococcus brevipes* (Cockerell), of which five were not present in the plant sap. It seems most probable that there is little or no uric acid, urea, allantoin, allantoic acid, creatin, or creatinin present in aphid honeydew [Lamb (23)]. Later we shall consider this peculiar circumstance under nitrogen metabolism.

The carbohydrates found in honeydew are given in Table II for several species of aphids and coccids. It is very difficult to compare the composition

TABLE II
CARBOHYDRATES IN HONEYDEW OF APHIDS AND COCCIDS

Carbohydrate	<i>P. brevipes</i> *	<i>C. hesperidum</i> †	<i>Cero-plastes species</i> ‡	<i>T. salignus</i> §	<i>Coc-coidea</i> 5 species	<i>B. bras-sicae</i> #
Fructose	+	+		+	+	+
Glucose	+		+	+	+	+
Sucrose	+	+		+	+	trace
Glucose-1-phosphate	+					
Maltose						
Glucosucrose		+				
Maltosucrose		+				
Maltotriosucrose		+				
Fructomaltose	+					
Ribitol			+			
Melezitose				+	+	0

* Gray (15).

† Wolf & Ewart (74).

‡ Hackman & Trokojus (17).

§ Mittler (36).

|| Ewart & Metcalf (11).

Lamb (23).

of honeydew with that of the plant sap since so few analyses are available. By paper chromatography, Duspiva (9) found that honeydew from the apple aphid, *Aphis pomi* DeGeer, and from the woolly apple aphid, *Eriosoma lanigerum* (Hausman), had a carbohydrate composition similar to the plant sap. Mittler (36) found only sucrose in the food, but glucose, fructose, sucrose, and melizitose in the honeydew of *T. salignus*. Ribitol was found in *Ceroplastes* sp. by Hackman & Trokojus (17). Ewart & Metcalf (11) were unable to find oligosaccharides in the host plant extract and, since Wolf & Ewart (73, 74) have isolated an enzyme containing fraction from honeydew of *C. hesperidum* which can induce the formation of melizitose and glucosucrose, it seems probable that most of the higher sugars present in honeydew are synthesized, possibly in the intestinal tract of the insect.

Studies of the excretory products of other insects have been less complete and usually were made to follow the path of a particular metabolite or to study the mechanism of the excretory process. Since Wigglesworth's (69) study of excretion in *Rhodnius prolixus* Stål, the best study of the excretory mechanism has been that of Ramsay (40 to 48) on the stick insect, *Carausius morosus* Bruner [= *Dixippus morosus* Bruner]. His work has a bearing on the composition of the excreta particularly as it permits, at least in *C. morosus*, following the material from the Malpighian tubes without contamination from other excretory material. This product of the tubes is called "urine" by Ramsay but it is not analogous to the urine, but rather to the glomerular fluid, of mammals. The tube fluid contains all the soluble, noncolloidal substances in the blood. If the concentration of any ion or molecule is temporarily increased in the blood by feeding or injection, more appears in the tube fluid. The composition of this fluid is then modified by resorption in the rectum (*Carausius*), and the remaining fluid, which is analogous to urine, is excreted. No great significance can be attached to the composition of the excreta unless the metabolism as a whole is considered. It is, however, important to discover the form in which material is excreted and to learn of any substances which are the end products of metabolism.

As an increasing number of insects are studied more carefully, we will find peculiar excretory products; for example, Harington (18) found that *Rhodnius* excretes histamine to the extent of 0.04 per cent of the dry excreta. *Rhodnius* blood has a very high histidine content, especially during the metabolism of a blood meal, and this is apparently decarboxylated before excretion. This conversion occurs in individuals free of symbiotes. Waterhouse (66) reported that the excreta of the webbing clothes moth, *Tineola bisselliella* (Hummel), contains 6 per cent cystine, but there is no evidence that it was ever present in the blood. Pteridines are present in the excreta of many insects, and in the milkweed bug, *Oncopeltus fasciatus* (Dallas), they constitute about 0.2 per cent of the total dry faeces [Hudson, Bartel & Craig (19)].

Toxic substances and the products of detoxifying reactions may occur in the excreta. Terriere & Schonbrod (58) have shown that a substance con-

taining ^{14}C is excreted by house flies for as long as 14 days after treatment with ^{14}C -labeled DDT. Piperonyl butoxide labeled with ^{14}C and administered to the Madeira roach, *Leucophaea maderae* (Fabricius), may be metabolized and excreted by the Malpighian tubes [Schmidt & Dahm (53)], but March and co-workers (29) suggest that in the American roach, *Periplaneta americana* (Linnaeus) the gut is the main region of metabolism and excretion for Systox. Topically applied nicotine is excreted by *Periplaneta* as cotinine [Guthrie *et al.* (16)].

Solid material in the products of the Malpighian tube is usually considered to be uric acid but a new and peculiar solid formation has recently been described. These particles, called "brochosomes," were first found associated with external structures such as wing scales or with the body surface by Tullock, Shapiro & Cochran (61), Tullock & Shapiro (62), Tulloch (63), and Wilde & Cochran (71). Day & Briggs (8) have shown that these granules form in the cells of the Malpighian tubes and appear on the surface of the insect as a contamination. They are apparently regular dodecahedra of unknown composition. They have been most often found on Homoptera.

NITROGEN METABOLISM

It has been well established [Wigglesworth (70)] that nitrogen is usually excreted as uric acid although some urea is generally present; in some insects, or during certain stages of development, ammonia may become a major constituent of the excreta. Unfortunately, our knowledge of nitrogen metabolism is still insufficient to give a clear picture but enough is known to have suggested certain lines of research that have proved fruitful.

The synthesis of uric acid in the fat body of the American roach has been established by McEnroe & Forgash (26, 27). They found that ^{14}C -labeled formate is incorporated into uric acid in positions 2 and 8. It is interesting that the ratio of ^{14}C in position 2 to that in position 8 was 2:1 *in vitro* and 1:1.4 *in vivo*. This ratio was explained by assuming an exchange of some ^{14}C in position 2 with inactive formyl carbon in the experiments *in vivo*. This is a good illustration of the caution needed in comparing experiments *in vitro* with *in vivo*. Their experiments also demonstrated that in the fat body the reaction glycine + formate \rightarrow serine occurs, which parallels the results of Sakami (51) with the rat. There does not seem to be any need to invoke the suggestion of Ludwig (24)—that uric acid is stored in the fat body to serve as a nitrogen depot—to explain the results McEnroe & Forgash (26) found in their *in vivo* experiments. Ludwig & Cullen (25) have shown that during starvation of the Japanese beetle, *Popillia japonica* Newman, the uric acid content of the blood does decrease, but they have no evidence for the destruction of the purine ring. It is very unlikely that, once formed, the purine ring will be broken in the insect. The uric acid metabolism in the mosquito, *A. aegypti*, has been followed by Terzian, Irreverre & Stahler (59). The uric acid content of the excreta of adult females on a sucrose diet decreased as shown in Table III. Analyses of the whole mosquito indicate that the nitro-

TABLE III

PERCENTAGE OF NITROGEN* IN THE EXCRETA OF *A. Aegypti*

	Duration of Sucrose Diet				
	Emergence	1st week	2nd week	3rd week	4th week
Total nitrogen	11.3	6.2	1.2	1.6	2.7
Uric acid nitrogen	9.3	1.9	0.2	0.2	0.1

* Dry wt.

gen content remains sensibly constant in the absence of protein intake. Irreverre & Terzian (20) have extended this work to include two other species, *Anopheles quadrimaculatus* Say and *Culex pipiens* Linnaeus, and made a more complete analysis. These mosquitoes were maintained on a 4 per cent sucrose diet and the excreta collected over a period of two weeks. Table IV shows the results. In the milkweed bug (*O. fasciatus*) fed on milk-

TABLE IV

THE DISTRIBUTION OF NITROGEN IN THE EXCRETA OF MOSQUITOES

Species	Percentage of total nitrogen				
	Uric acid	Urea	Ammonia	Protein	Amino Acids
<i>A. aegypti</i>	47.30	11.90	6.40	10.82	4.40
<i>A. quadrimaculatus</i>	42.50	9.50	7.80	9.22	4.70
<i>C. pipiens</i>	46.90	7.90	10.00	9.07	5.50

weed seeds, uric acid comprises 3.0 to 3.5 per cent (dry wt.) and pteridines 0.2 per cent of the total fecal material. The precursor of the pteridines is not known [Hudson, Bartel & Craig (19)].

In *Sialis lutaria* (Fabricius) larvae, Shaw (55) and Staddon (56) have shown that ammonia constitutes about 90 per cent of the nitrogen excreted during starvation. The total ammonia concentration in the body is 0.001 per cent (wt./wt.) and in the contents of the hind-gut 0.165 per cent (wt./vol.). Nitrogen is excreted at the rate of 10 μ g. N per 100 mg. (wet wt.) per day. A concentration of 8.5 mg. NH_3 per 100 ml. blood is toxic. If excretion is prevented there is no accumulation of ammonia, and it may be transformed into some other nitrogenous compound.

Support for the existence of the Krebs-Henselet cycle as the mechanism for urea formation in insects is furnished by the finding of free ornithine and arginine in all stages of development of several species of Lepidoptera,

Hymenoptera, and Orthoptera by Garcia, Tixier & Roche (12). Citrulline was present in some of the stages. Experiments *in vitro* showed arginase activity. The authors' conclusion that "without doubt the urea is utilized for formation of uric acid" is unsupported.

Amino acids are not generally found in the excreta of most insects unless they happen to be present in the blood in great excess. The urine from the Malpighian tubes, at least in *C. morosus*, contains amino acids [Ramsay (48)] which are removed in the rectal region and returned to the blood. Since evidence of tube uptake of amino acids had been found previously by Patton & Craig (39) for *Tenebrio molitor* Linnaeus, it may eventually be found that the urine of many insects contain amino acids. It is only in the Homoptera that we find amino acids excreted in large amounts and forming the typical nitrogenous waste. While uric acid has been reported present in the honeydew of the citrus mealybug by Gray & Fraenkel (14), no uric acid was found by Maltais & Auclair (28) in the honeydew from *M. circumflexus*. In honeydew from *B. brassicae*, Lamb (23) found no uric acid, urea, allantoin, allantoic acid, creatine, or creatinine. Ammonia may be present in the 5 to 8 per cent of undetermined nitrogen. It is probable that, at least in these two aphids, nearly all the nitrogen is excreted as amino acids. Lamb suggests that a possible function of the bacteroids in these insects is to remove the ammonia produced since bacteria known to fix nitrogen frequently prefer ammonia as a nitrogen source. There is, however, as yet no good evidence that the bacteroids are actually capable of fixing nitrogen or even ammonia as Toth (60) has repeatedly suggested. Mittler (37) developed the technique of obtaining the actual material ingested by the aphid by cutting the inserted stylets near the head. The plant sap continues to flow from the severed stylets for many hours and may be collected in micropipettes. This makes possible the determination of the nitrogen assimilated by the aphid as the difference between that ingested and that excreted in the honeydew. Using this method Mittler carefully studied the nitrogen balance of *T. salignus* feeding on willow. He found that the increase in nitrogen content of the aphids could be accounted for on the basis of plant sap and honeydew and that no evidence for fixation of atmospheric nitrogen existed in his experiments. The aphids passed more sap through their intestinal tracts on a nitrogen-poor sap diet than on a rich diet. During reproduction the aphid contributes some stored nitrogen from her body. The rate of reproduction is greater in the alate form (about three young a day), so that the body weight of the alate is reproduced in about five days. From Table I it may be determined which amino acids are used by *M. pisi* during the reproductive period as ascertained by Auclair (1).

These results of the nutritional and excretory experiments with aphids tempt one to speculate. If the aphid has access to an abundance of all the required amino acids so that synthesis is not a limiting factor in maintaining the amino acid balance in the blood, then why should uric acid or urea be formed? There would be some breakdown of purines but such endogenous

uric acid might be too negligible to have been detected, as could also be true of ammonia. There is an interestingly similar situation in the case of the protozoan *Tetrahymena pyriformis* (Ehrenberg). This animal has been extensively studied and its nitrogen balance has been reviewed by Gordon (13). It has been shown that, on an abundant diet, amino acids are absorbed and excreted to maintain a balance. With poor diets much synthesis must occur and ammonia is produced. Aphid excretory products might be different if the diet were poor.

THE MALPIGHIAN TUBES

No attempt has been made to find all the reports of the gross anatomy of the Malpighian tubes or their disposition in relation to the gut. Attention is called, however, to such a study by Maxwell (33) in the case of sawflies. In her study of the internal larval anatomy of 132 species, she has shown the structure and position of the Malpighian tubes. There is much variation from the simple free type to those attached (perhaps functionally?) by their distal end to the rectum or even to the mid-gut. Unfortunately, the sawflies are poor laboratory animals. The study by Savage (52) of the origin and development of the tubes of *Schistocerca gregaria* Forskål is of considerable morphological interest. The six primary tubes arise as outgrowths of endodermal origin which develop into a tube formed of a simple epithelium. The differentiation of the tube into a recognizable proximal and distal region, with the development of a striated border, does not occur until after hatching, at which time the tubes become functional. During embryonic and nymphal life many more tubes arise at each molt until the average number of tubes in the adult is 250. The initiation and growth of a set of tubes in one instar is followed by their differentiation and functional development in the next instar. Miles (35) has shown that in *Oncopeltus* there is no opening between the mid- and hind-gut until the adult stage. In the nymphal instars all the excreta must come from the Malpighian tubes and perhaps the hind-gut.

The cells of the Malpighian tubes have been studied from several points of view. The most interesting from a purely structural standpoint are those of Beams, Tahmisian & Devine (3), Berkaloﬀ (4, 5), Bradfield (6), and Meyer (34) because, made with the electron microscope, they help interpret such fine structures as brush borders. Beams *et al.* used the Malpighian tubes from the grasshopper *Melanoplus differentialis differentialis* (Thomas) fixed and sectioned by a standard procedure. These tubes were composed of a single layer of cells resting on a basement membrane. For descriptive purposes the portion of the cell next to the basement membrane is called the basal, followed by the intermediate and apical zones. The basal zone is divided into many compartments by deep, parallel infoldings of the cell membrane. These compartments range from 38 μ to 160 μ in width; they contain, among other things, one or more mitochondria. There may be interdigitation of these compartments in adjacent cells. The intermediate zone contains

the relatively large nucleus with a well-defined nuclear membrane. The cytoplasm contains many short rod or filamentous mitochondria having numerous double membranes. This inner structure becomes more pronounced as the apical zone is approached. The apical zone, which borders the lumen of the tube, bears the prominent brush border. This border is composed of many parallel protoplasmic processes perpendicular to the tube axis which are 1μ wide and 3 to 4μ long. When filled with mitochondria, these processes are distended and bulb-shaped at the distal end. It is conjectured by Beams *et al.* that the mitochondria flow into these processes and eventually are discharged into the lumen of the tube by the pinching off of the bulbous end. Bradfield (6), in a preliminary study of the Malpighian tubes of the wax moth *Galleria mellonella* (Linnaeus), also saw mitochondria in these positions but did not assume that they were about to be discharged from the cell.

Meyer (34) made a comparative study of the Malpighian tubes of the stick insect, *C. morosus*, the honey bee, *Apis mellifera* Linnaeus, a bumble bee, *Bombus* sp., and the oriental roach, *Blatta orientalis* Linnaeus. In *Carausius* the secretion of the cells consisted of numerous intracellular granules. These were compacted in the lumen to form elliptical bodies. The mitochondria are appressed to the distal end of the processes of the brush border as described by Beams *et al.*, but evidence of actual discharge into the lumen could not be found. In *Apis* small intracellular inclusions were observed in the cytoplasm. The brush border is very voluminous but the mitochondria were not seen in them. In *Bombus* there were also many inclusions of a characteristic structure, and they, with the mitochondria, seemed to be erupting through tears in the brush border into the lumen of the tube. It is possible that the mitochondria are encapsulated in the inclusions. In *Blatta* the relationships are similar to those in *Bombus* except that the intracellular inclusions have the same shape as the extracellular granules in *Carausius*. The author concluded that in none of the cells studied was there Golgi apparatus of the type described for vertebrates or insects.

Berkaloff (4) stated that the cells of the Malpighian tubes of the cricket, *Gryllus domesticus* Linnaeus [= *Acheta domestica* (Linnaeus)] have the same fine structure as those of the proximal loop of the renal tubule of vertebrates. There exists the same system of invaginations with double membranes. In the cells of *A. domestica* there are two types of granules, the yellow ones which give the tubes their color, and the other type composed of concentric shells of greatly differing electron opacity. These grains are 1.5μ in diameter and give the murexide test. They arise in a complex of double membranes in the periphery of the cell, grow by accretion, and migrate toward the central cytoplasm by rupture of the enveloping membranes. They are eliminated into the lumen of the tube all at one time for each cell and are no longer visible in the lumen. This disappearance Berkaloff considered to be probably a fixation artifact. Similar grains were found in *Bombus* and in a carpenter bee, *Xylocopa* sp. In a later paper Berkaloff (5) reported the β -cytoplasmic mem-

branes in the Malpighian tube cells of *Acheta* to be completely similar to those found in the vertebrate kidney tubule loop cells by Rhodan (49). He reports passage of urate granules (identified by the murexide test?) from one part of the cell toward the lumen by rupture of the β -cytoplasmic membranes.

Using Rigaud's technique, Monteiro (38) has briefly studied the chondriosomes and mitochondria of the drug store beetle, *Stegobium paniceum* (Linnaeus). The number and positions of these bodies agree with the results of Beams.

The granules described by electron microscopists have not been correlated with those described by use of the light microscope. Thus Day (7) described phosphate granules in the cytoplasm and striated border of the tube cells and in the lumen of the Malpighian tube of the fly *Lucilia cuprina* (Wiedemann) and in some of the tube cells in *Periplaneta*, *Apis*, and several other insects. Waterhouse (64, 65) discussed the granules in the Malpighian tube cells of *Lucilia* and showed that they contain barium and strontium and are soluble in some histological fixatives, thus resembling Berkaloﬀ's fixation artifacts. This reviewer does not feel competent to evaluate descriptions of cell structure, but it seems certain that many revisions in parts of the present pictures are inevitable.

The histochemical studies of Malpighian tubes have included several on enzymes. Mazzi & Baccetti (30, 31) found phosphatase present in both the proximal and distal part of the tubes in *Donus crinitus* Boheman, although the distal part produces silk and the proximal part is concerned only with excretion. The proximal part of the tube also elaborates a periodic acid-Schiff-positive substance which is not glycogen. In a later paper Mazzi & Baccetti (32) discussed the comparative histochemistry of the tubes of *Blaps gibba* Costelnau, *Mantis religiosa* Linnaeus, *Acrida bicolor* Thunberg, and *Apis* with respect to enzyme activity and localization. Their results are in Table V. It can be seen that there is a marked difference in enzyme distribution and little correlation with known function. Evans (10), using the reduction of several tetrazolium salts to colored formazans as indicators, studied

TABLE V
THE DISTRIBUTION OF ENZYMES IN MALPIGHIAN TUBES

Enzyme	<i>A. bicolor</i>			<i>M. religiosa</i>			<i>B. gibba</i>			<i>A. mellifera</i>		
	p	i	d	p	i	d	p	i	d	p	i	d
Alkaline phosphatase	+++			++			+++			+++		
Acid phosphatase	++	++		++			+++			++	++	++
Lipase		++		++				++	++	++		+++
Succinic dehydrogenase	+++	++	+	++	+		+	++		++	+	

p = proximal, i = intermediate, d = distal region of tube.

the localization of dehydrogenase activity in several tissues associated with the gut of larval *Calliphora erythrocephala* (Meigen). He found regions of intense coloration because of formazan and regions of no coloration with little uniformity among different specimens.

The enzyme systems have been studied by biochemical methods which can yield results of considerable interest. Auclair (2) has studied the amino acid oxidases present in the fat body and Malpighian tubes of *P. americana*, *G. mellonella*, the German roach, *Blatella germanica* (Linnaeus), and *O. fasciatus*. The homogenate of the roach tubes actively produced keto acids on incubation with DL- α -amino-butyric acid, DL-aspartic acid, D- and DL-methionine, and D- and DL-norleucine. Other amino acids were less rapidly oxidized and DL-homocystine, DL-isoleucine, L-leucine, and DL-proline were not oxidized. Methionine, norleucine, and leucine were not oxidized by the tube brei of the wax moth or the milkweed bug, but methionine and norleucine were oxidized by the brei of German roach tubes while leucine was not. Transaminases are present in *S. gregaria* tubes [Kilby & Neville (22)] and in *P. americana* tubes and rectal regions.² Homogenates of the grasshopper tubes catalyzed the aspartate-glutamate and alanine-glutamate transaminations. The enzymes are present in both the soluble and mitochondrial fractions of tube cells. In the milkweed bug the same reactions are catalyzed.

There are reports of other substances being present in Malpighian tubes although frequently no particular significance can be attached to their presence. Ishihara (21) has shown that riboflavin accumulates in the tubes of the silkworm larva, as much as 1.8 μ g. per mg. of fresh tissue occurring in the 11-day-old, fifth-instar individuals. The *od* strain of silkworms show very much less accumulation. Another case of genetic influence is shown by the rosy mutant of *Drosophila melanogaster* Meigen. Schwinck (54) found this form to excrete a red pigment in the Malpighian tubes. Implantation of the tubes increases the amount of eye pigment. It is known that pteridines and other fluorescing substances are often found in the tubes. An experimental survey of the fluorescence of the Malpighian tubes of a number of species of Blattellidae has been made by Willis & Roth (72), but no information was given as to the nature of the substances present.

THE MECHANISM OF EXCRETION

Although an enormous amount of effort has gone into the study of excretion in animals, we still do not fully understand the excretory processes of any organism. However, we are continually adding to our store of facts and we learn by appreciating first the differences and then the similarities of functioning systems. Perhaps one of the difficulties in the study of the insect excretory system was the assumption that the Malpighian tubes formed a complete excretory system, a view which places upon them the entire burden of water and osmotic pressure regulation as well as the maintenance of specific levels of concentration for all the multitude of blood components. One

² Unpublished experiments of C. L. Judson and R. Craig.

previous correction of this belief was given by Patton & Craig (39) but this was not enough to alter established habits of thought. The mechanism of excretion as suggested by them has recently been reiterated and supported by an imposing mass of data gathered by the most advanced methods in a careful and painstaking series of experiments by Ramsay (40 to 48).

Ramsay (40) showed that in mosquito larvae the osmotic regulation is aided by the excretory system. When the external medium is low in salts the urine is lower in sodium than the blood. It is not possible for the tubes to form a urine higher in sodium content than the blood. Ramsay (41) then turned to *Rhodnius* and studied the formation of urine and its alteration as it passed from the distal to proximal region. By injecting potassium into the blood he was able to show that this insect can remove potassium from the blood against a concentration gradient. The urine in the distal portion of the tube contains more potassium but less sodium than the blood. In the proximal region the urine becomes more nearly like blood in its concentration of sodium and potassium. Ramsay assumed that sodium and water were probably resorbed in the rectal region. These results were then compared with the situation in the larva of *A. aegypti* [Ramsay (42)]. Here is a system in which the concentration of, at least, sodium and potassium can be altered in a physiological manner. By increasing the concentration of salts in the medium bathing the larvae, the concentration in the blood is increased by resorption through the anal gills. The concentrations of both sodium and potassium increase in the urine with increased blood concentration and the absorption in the rectum decreases. There is a circulation of potassium from the blood, to the tube, to the gut, and back to the blood. The concentration of potassium is always greater and sodium less in the urine than in the blood. The volume of the larvae seem to be regulated by the rate of voiding which, of course, affects the amount of resorption. This rate is controlled by the peristaltic action of the gut. Ramsay's study was then generalized to eight species of insects: the migratory locust *Locusta migratoria* Linnaeus; the stick insect *C. morosus*; the European cabbageworm *Pieris brassicae* (Linnaeus); the beetle *Dystiscus marginalis* Linnaeus; the yellow mealworm *T. molitor*; a *Tabanus* sp., the yellow-fever mosquito *A. aegypti*; and *Rhodnius* [Ramsay (43)]. At least in these species the concentration of potassium is always greater in the urine than in the blood. In addition, the electrical potential across the wall of the Malpighian tube, as measured by silver chloride electrodes, indicated an accumulation of positive charges in the lumen of the tube. Since the sodium concentration was usually less in the tube, Ramsay concluded that, while potassium is actively secreted into the tube, sodium simply diffuses in. In an appendix to this paper Ramsay describes his technique for obtaining samples of urine direct from the tube by inserting a finely pointed silica tube.

Ramsay (44) next turned to the stick insect, *C. morosus*, for more detailed observation, and in a series of finely executed studies from 1954 to 1958 he has shown in this insect a probable mechanism of excretion which can serve as a model upon which to base future studies. In the stick insect the

urine is *iso*- or slightly hypotonic, compared to the blood. The rate of urine formation is about 7×10^{-6} mm.³/mm.²/min., which, when calculated for the system, amounts to about 6 mm.³/hr. or a volume equal to the blood volume, filtered in 24 hr. The Malpighian tubes of this insect [Ramsay (46)] actively secrete sodium, potassium, and water in all regions, but the Na/K ratio is greater in the proximal region. Potassium acts as a diuretic, but sodium has little effect. The rate of secretion of potassium is more than tenfold that of sodium. Shaw (55) showed that a similar situation exists in *Sialis* larvae where potassium is removed from the blood more readily than is sodium. The blood of *C. morosus* is very high in magnesium and, in fact, could be considered saturated with magnesium phosphate. [Ramsay (45)]. It should be remembered that in all these studies of biological fluids, concentration is given as total concentration and cannot be interpreted as ionized or unionized, free or bound. It is unlikely that magnesium is present only as the salt. Calcium, magnesium, chloride, and phosphate are present in the urine [Ramsay (47)]. Of these, all are at a lower concentration in the urine except phosphate, for which the urine concentration is greater than that of the blood. The rate of urine flow is proportional to the phosphate concentration of the blood. The urine is alkaline compared to the blood but becomes acid in the rectum. In his most recent paper, Ramsay (48) presents an extended study of the excretory process of *C. morosus* with respect to organic substances. The substances included the amino acids alanine, arginine, glycine, lysine, proline, and valine; the sugars glucose, fructose, and sucrose; and, as a substance frequently studied in mammals, urea. These substances, isotopically labeled, were added to a saline solution. A Malpighian tube, dissected from the insect, was arranged on a microscope slide so that a portion of it passed through a drop of the saline. The tube takes up or secretes a portion of the material in the drop and the radioactivity appears in the urine produced. The urine can also be chromatographed and the identity of the substances and their concentration determined. Some substances, e.g. valine, are metabolized on passage through the tube cells as shown by the presence of radioactivity in several spots. In all experiments the concentrations of inorganic salts were lowered to maintain isoosmotic conditions and experiments were run with different ions at low concentration to rule out specific ion effects on the excretion of the organic substances. The results are very significant. The *U/P* ratio (concentration of substance in urine/concentration of substance in blood) is nearly independent of the concentration in the saline. There does not appear to be any maximum or minimum (threshold) concentration for the removal of amino acid from saline by the tubes. The *U/P* ratios are different for the several amino acids, being highest (0.6) for proline. The excretion of the sugars and urea was studied in the same way as that of the amino acids. The *U/P* ratios for the sugars were about 0.6, depending slightly on the concentration; the *U/P* for urea was 1.0. All these results are in accord with the idea that these substances pass through the walls of the tube by diffusion. To test this hypothesis Ramsay perfused the tube with a saline and

measured the rate of uptake of urea and sucrose as a function of the perfusion rate. The permeability of the tube of *C. morosus* to urea is among the highest measured for natural membranes. To summarize these studies we may assume: (a) The Malpighian tube is freely permeable so that most metabolites of small-sized molecules pass through under the influence of the concentration gradient. (b) Some salts and water are actively secreted. It is not clear whether there is interaction between water and the salts. (c) This stream of saline passes rapidly down the tube and flushes out the diffusing substances. (d) The substances required by the insect are absorbed by the rectum and returned to the blood. (e) As discussed by Ramsay, it is simpler for an organism to develop ways of absorbing needed substances than to develop ways of excreting all the possible undesirable ones.

THE FUNCTION OF THE INTESTINE IN EXCRETION

The rectal region of the hind-gut has not been critically studied either histologically or physiologically since the advent of the newer methods so effectively used on the Malpighian tubes. Reichenbach-Klinke (50) has examined many species for the presence of rectal papillae and no doubt other descriptions are available hidden in the anatomical literature.

Waterhouse (68) has described the differentiation of the hind-gut of *L. cuprina* larvae into longitudinal bands of histologically differentiated cells. He finds no evidence for excretion of ammonia by the Malpighian tubes and assumes that active deamination is carried out by the mid-gut epithelium, the ammonia passing into the blood and being removed from the blood by the hind-gut epithelium. It would be interesting to investigate this situation using biochemical methods.

STORAGE EXCRETION

By our definition of excretion, any method of removing a substance from metabolic activity becomes a process of excretion. There are many well-known examples in both vertebrates and insects of this storage excretion and new examples are continually arising. In the webbing clothes moth the goblet cells of the mid-gut concentrate and store heavy metals as the sulfides [Waterhouse (66, 67)]. Uric acid is repeatedly reported as present in crystal or granular form. It is unfortunate that it is so difficult to identify this substance by histochemical methods. Recently Yamafuji (75) has isolated uric acid from nuclear preparations from silk worms and identified it by paper chromatography and absorption spectra. Stich & Grell (57) have shown that *C. pipiens* tubes retain the phosphorus resulting from dissolution of larval tissue in the Malpighian tube cells for the duration of metamorphosis.

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CYTOGENETICS OF INSECTS^{1,2}

By S. G. SMITH

Cytogenetics Section, Forest Biology Laboratory, Sault Ste. Marie, Ontario, Canada

The subject of cytogenetics, even when limited to insects, is immense. To cover it comprehensively in the allotted space is impossible. Fortunately, population genetics and chromosomal polymorphism in *Drosophila* have recently been reviewed by da Cunha (17) and Stone (81) respectively, other topics are covered in this volume by da Cunha and by Laven, and cytogenetics as a whole and of Orthoptera in particular have been thoroughly treated by White (93, 97, 98) on several occasions. The present article will consequently be restricted to recent highlights viewed, where possible, in relation to published and unpublished findings in the Coleoptera. It is assumed that White's (98) contribution in this series of *Annual Reviews* is known to the reader, and the terminology adopted herein is the same as in that article.

CHROMOSOME MECHANICS

In certain groups of bisexual insects, such as the Diptera and Homoptera, the "normal" sequence of events leading to the halving of the chromosome number has been profoundly changed by the development of new mechanisms. Comparative analysis of these diverse systems, besides elucidating broad problems in taxonomy and evolution, throws considerable light on the cytogenetic principles governing chromosome behavior.

Certain groups in the orthopteroid complex include species in which chromosome pairing in the male appears to be maintained to the end of prophase in the absence of visible chiasmata. In summary, homologues retain a close parallel association from zygotene to prometaphase or metaphase, so that typical diplotene and diakinesis are omitted; with dissolution of the nuclear membrane, the bivalents, roughly orientated up and down the developing spindle, may either be torn open in the region of the centromeres or the intimate association may persist until anaphase; in the former, separation at the centromeres proceeds rapidly to the ends of the chromosomes, drawing out the bivalents in a premetaphase stretch; the stretching then relaxes and the bivalents congress to form the metaphase plate.

These anomalies are by no means restricted to the orthopteroid insects. Their importance to cytogenetic theory is attested to by the diversity of opinion expressed on their causal relationship, as well as by the recent resurgence of active interest in them. In the case of *Periplaneta americana* (Linnaeus), John & Lewis (37) have shown by the use of spindle-inhibiting agents that the protracted side-by-side pairing of homologues results from a

¹ The survey of literature pertaining to this review was concluded in May, 1959.

² Contribution No. 561, Forest Biology Division, Research Branch, Department of Agriculture, Ottawa, Canada.

nonspecific matrical stickiness, which ends at prometaphase. The absence of diplotene and diakinesis they attribute to the intervention of the stretch, and suggest that "whilst chiasmata may be formed in *P. americana*, matrical stickiness and terminal affinity are the main factors which maintain the association of homologues." Callan & Jacobs (13), however, state categorically, "... we are convinced that the chromosomes of *Mantis religiosa* are associated by chiasmata at meiosis," and they consider that apparent differences between meioses in the orthopteroid group are only superficial.

A mechanically unique basis for the apparent absence of chiasmata in blattids is proposed by Piza (56)—an interpretation that is obviously influenced by his theories of chromosome structure and behaviour in Hemiptera and other groups. He asserts that no chiasmata are formed in any of the seven species of Brazilian roaches he studied. The essence of Piza's argument is as follows: (a) the autosomes split into sister chromatids before pairing; (b) sister centromeres repel one another, thus moving the chromatids apart except at the end of the longer arm; (c) homologous (non-sister) chromatids pair; (d) progressive repulsion, initiated centrically, eventually separates the shorter arms, and the bivalent then consists of homologous chromatids associated side by side; (e) the first division is thus equational, the second reductional.

Piza's scheme is completely at variance with the observations of other authors. Moreover, this theory is invalidated by Piza's own description of an unequal bivalent in *Epilampra verticalis* Burmeister. Instead of concealing its size inequality at metaphase I, as it should if meiosis is postreductional, the heteromorphic pair is always conspicuous and its division is consequently invariably prereductional.

John & Lewis's (39) interpretation of the course of meiosis in the roach *Blaberus discoidalis* (Serville) is, as expected, diametrically opposed to that outlined above. It is neatly verified by this roach's possession of procentric blocks of heterochromatin that reveal the conventional nature of its synapsis. Although differences may of course exist between the genetic systems of even quite closely related species (see later), it is difficult to find justification for Piza's claims for the Brazilian roaches.

The chromosomes of Homoptera, Hemiptera, and Lepidoptera have been generally regarded as having nonlocalized centromeres: they are considered to be polycentric or to possess a dynamic activity diffused over their entire length. Recently the anopluran *Haematopinus suis* (Linnaeus) and the mallophagan *Gryopius ovalis* Burmeister have been included in this category [Bayreuther (5); Scholl (66)], but some Heteroptera and Homoptera-Auchenorrhyncha are now known to have localized centromeres [Dutt (19); Rao (58)].

Piza (57) is alone in maintaining the unorthodox idea that the chromosomes in the Heteroptera are dicentric. They are believed by Piza to consist of two chromatids terminally conjoined in a reverse tandem arrangement whereby the two centromeres are situated at opposite ends of the joint

chromatids. Piza refers to such chromosomes as isochromosomes, but such usage is confusing. Piza's concept of dicentricity here and elsewhere has yet to receive independent confirmation. It is noteworthy in this connection that the chromosomes of *H. suis* and *G. ovalis*, which arc with concave side toward the poles at anaphase, superficially fit into Piza's scheme. Bayreuther (5), however, has demonstrated that radiation-induced fragments in *H. suis* have the same crescentic shape in anaphase and hence considers them to be "holokinetisch."

Tuzet & Manier's (86) claim that meiosis in the Collembola is initiated in octoploid cells and that haploid gametes result from a series of reduction divisions is completely unsubstantiated by Saure & Brummer-Korvenkontio's (65) findings.

The X and Y chromosomes of tipuloid Diptera associate either by somatic pairing or by the nonspecific adhesive properties of their heterochromatin. They separate under the influence of a premetaphase stretch or earlier but subsequently move to the equator [Dietz (18)]. From here they either segregate regularly (by some unknown means), in which case the second division is equational, or they divide equationally, as in *Tipula maxima* Poda, and segregate at the second anaphase. In *T. maxima* the X and Y chromatids pair, not necessarily specifically, during second prophase as they become "recharged" (nucleinated), their centromeres co-orientate, and active mobility in the "undercharged" ends, reinforced by active repulsion of the procentric heterochromatin, effects their disjunction [John (36)]. One end of each of the X and Y thus shows neocentric activity, a property they share in lesser degree with the ends of the autosomes. Obviously, if all the ends behaved uniformly in this respect, a condition would be presented paralleling that described in Heteroptera [Piza (57)].

Comparable sequences of division exist in certain hyperaspine coccinellids. North American *Hyperaspis* species have X and Y chromosomes that behave as do those of *T. maxima*, or it might be said that they follow the heteropteran postreductional sequence of division [Smith (73)]. According to S. G. Smith (unpublished data), *Hyperaspis billoti* Mulsant var. from Trinidad, although resembling *maxima* in having a premetaphase stretch, is like the Neuroptera in showing distance pairing and precessional disjunction [as also in Mecoptera, see Cooper (16)].

Le Calvez (41) found the mycetophilids to comprise yet another in the diverse array of cytogenetic systems that distinguish the Diptera. Although entirely lacking chiasmata, the X and Y come to lie indiscriminately within the equator, not vis-à-vis across it, they nevertheless achieve a regular, albeit belated, segregation. Some 50 years ago, Stevens (80) briefly described a strikingly similar case in a beetle, *Altica chalybea* (Illiger); her observations have been confirmed in other *Altica* species by Smith & Manna (unpublished material).

It is consequently of interest to find in the Coleoptera the independent origin of anomalous meiotic mechanisms found in the Orthoptera, Diptera,

Neuroptera, Mecoptera, Heteroptera, and—through the postreductional X of lampyrids [Smith & Maxwell (76)]—the Odonata. This must surely caution against inferring broad phylogenetic significance from joint possession of apparently aberrant cytological features.

Chiasmate meiosis and crossing-over occur in *Culex*. Despite the considerable body of evidence that, where a sex differential in crossing-over exists, it is the heterogametic sex which shows the lesser amount, Laven (40) finds crossing-over in *Culex* apparently to be confined to the male. Presumably chromosome association in oögenesis is maintained by somatic pairing, the reverse of what prevails elsewhere in the order.

Wolf (103) finds that the frequency of crossing-over and the allocyclus of the X in the fly *Anisopus cinctus* (Fabricius) are temperature dependent. It is conceivable that the disproportionately high chiasma formation within negatively heteropycnotic regions described by Virkki (88) in the beetle *Agriotes mancus* (Say) may be similarly explained.

SEX-DETERMINING MECHANISMS

Because it is generally agreed that the XX ♀:XY ♂ system is more primitive than the XX ♀:XO ♂, it is therefore reasoned that neo-XY mechanisms are evolutionary reversions from a prior XO condition.

The reversion from XO to XY in the phasmid *Isagoras schraderi* Rehn, in which both sex chromosomes are submetacentric, becomes intelligible in the light of conditions in *Chilocorus*. Hughes-Schrader (29) assumed two successive reciprocal translocations between an original V-shaped heterochromatic X and each of a pair of autosomes, resulting in X and Y chromosomes in which the eu- and heterochromatin are largely restricted to separate arms of each. If, however, the sex-determining genes were confined to one arm of the X and if the autosome concerned had an entirely heterochromatic arm (as is probable since congeneric species possess a heteromorphic pair), all that need be postulated is a simple centric fusion of the type seen in *Chilocorus hexacyclus* Smith (74). Admittedly the pairing arms in *I. schraderi* are of different size, but this may well result from subsequent change [cf. various mantids, see Hughes-Schrader (30)].

A survey of the Coccinellidae establishes that the neo XY of the Chilcorini had its origin, as the term neo XY implies, in the fusion of the X with one of a pair of autosomes. Moreover, proof that the XXY system in *Chilocorus stigma* Say arose by a second fusion that united a neo Y with a member of still another autosomal pair, as occurred also in the evolution of *Paratylotropidia morsei* Rehn & Rehn and *P. brunneri* Scudder [White (94)], is provided by reciprocal hybrids between *C. stigma* and *C. tricyclus* Smith (or *C. hexacyclus*). F₁ males from *stigma* ♀ × *tricyclus* ♂ possess a neo XY; those from the reciprocal, a synthetic X₁X₂Y₁Y₂ complex in which the Y of *stigma* is paired with one of the fused autosomes of *tricyclus*, which in turn is paired with a *stigma* autosome.

Unlike the morabine grasshoppers [White (98)], in which the neo-XY condition seems to be a mere steppingstone from an XO to an XXY system, in beetles the neo XY is stabilized in almost 90 of the 700 known species (S. G. Smith, unpublished findings). In fact, excluding *C. stigma*, multiples are found only in the Cicindellidae [Guénin (23); Smith & Edgar (75)], the Blaptinae [Guénin (24, 25)], and a single chrysomelid [Gôto & Yosida (22)]. Moreover, apart from that in *stigma*, the system in the chrysomelid seems to be the only orthodox one. Lewis & John (43) consider polyploidy and possibly hybridization as factors in the evolution of the sex multiples in *Blaps*.

The Heteroptera are characterized by the stereotyped postreductional behaviour of their sex chromosomes during spermatogenesis [Manna (45)]. Major exceptions have been recorded in two pyrrhocorids, *Dysdercus koenigii* (Fabricius) and *D. mendesi* Bloete [see Manna (45)], in the first of which the X and Y show double reduction and in the second undergo a double equational division. By such mechanisms half the spermatozoa in the former species would come to lack both sex chromosomes, whereas in the latter all would carry both, as in *Drosophila* males carrying the sex-ratio gene. Re-investigations cited by Manna (45) have elucidated the situation: the mechanism in both species is XXXX ♀ : XX ♂ and postreductional as elsewhere in the order.

Using British midges, Acton (1, 2) has confirmed Beermann's (6) salivary gland demonstration of the existence of sex chromosomes marked by inversions in German and Swedish *Chironomus tentans* Fabricius and *Chironomus annularius* Meigen, and he has extended his investigations to include three additional species. A comparable situation is found to exist in the Simuliidae by Rothfels (61).

CHROMOSOMAL POLYMORPHISM

Although White (97) was led to state, in 1957, "Natural populations which are polymorphic in respect of centric fusions are not common," an impressive number have come to light in recent years. The discovery and study of such populations is of vital importance, for a comparison of their chromosomal diversity with the karyotypes of related species may be expected to provide revealing evidence as to their potential evolutionary value. By the same token, the detailed comparison of sibling or other closely related species will often disclose which adaptive polymorphism—which genetic mechanism—has been tried and found successful.

Ohmachi & Ueshima (53) have described a puzzling case of variation in the grylloid *Euscirtus hemelytrus* de Haan that they were unable to reconcile with known exceptions to the principle of numerical and morphological constancy of the karyotype. If, however, it is assumed that *E. hemelytrus* is basically $2n\sigma = 18 + X$, as in other species, the distribution pattern of univalents at metaphase suggests premeiotic nondisjunction, floating super-

numeraries, and asynchronous precocity. Other chromosomally polymorphic crickets have recently been reported [Bergerard (8); Ohmachi & Ueshima (52); Ueshima (87)].

Waterhouse & Sanderson (92) have placed on record the first case of chromosomal polymorphism known in the Hymenoptera. Although several males and females of *Tenthredo acerrima* Benson had 18 and 36 chromosomes, members of one brood had 21 and 42. There does not appear to be any obvious reason why hymenopteran chromosomes should be exempt from the types of structural change commonly encountered in other orders. In fact, the absence of chiasma formation in the haploid males should predispose them to such changes. The failure to detect polymorphism earlier is most reasonably ascribed to technical difficulties, to the restriction of synaptic meiosis to females, and to the need for large-scale surveys. Thus Halkka's (26) recent investigation of 121 species of Homoptera-Auchenorrhyncha has revealed a case of polymorphism for a neo-XY vs. XO condition.

The eastern North American *Chilocorus stigma* is polymorphic for three autosomal fusions and for up to five supernumerary chromosomes [Smith (73, 74)]. Where monomorphic, the female has 26 metacentric chromosomes including 4 X's, 2 of which are replaced in the male by a metacentric Y. In increasingly complex polymorphs, pairs of nonhomologous metacentrics become replaced by single superficially similar metacentrics, with a consequent downward progression in number. This "Robertsonian" imbalance is rendered possible, without recourse to pericentric inversion, by the dual nature of the unfused chromosomes, or "amphisomes," wherein one arm is totally heterochromatic, the euchromatin being restricted to the other. Chiasma formation is limited to euchromatin; hence the wholly heterochromatic by-products are either eliminated or float in the descendent population as supernumeraries.

Polymorphism in the North American weevil genus *Pissodes* is widespread at both the intra- and interspecific levels [Manna & Smith (46)]. Three polymorphic species each carry two or three floating metacentrics, similar to those present in the lower-numbered monomorphic species. Superimposed on this variability there is a further, unique structural polymorphism in the form of a centric transposition. The shift has converted a J-shaped chromosome into a structurally different but morphologically indistinguishable metacentric, which when heterozygous forms an asymmetrical ring and when duplicated constitutes an "isomer" of the original uninverted ring. Moreover, both metacentrics may occur as their corresponding twin acrocentrics. A surprising feature of these polymorphs is that all those, including the isomers, known in *Pissodes approximatus* Hopkins and *Pissodes canadensis* Hopkins from Ontario and Manitoba are potentially present also in the California *Pissodes terminalis* Hopping.

Centromere transpositions from a quasi-terminal to an interstitial position are relatively common polymorphisms in North American trimerotropine grasshoppers and occur also in the reverse direction in the Australian

eumastacids [White (95)]. Despite the prevalence in the Orthoptera of species monomorphic or polymorphic for centric fusions or fissions, such metacentrics, as in the Coleoptera, seem to have been almost exempt from subsequent pericentric inversion; at least they have only rarely persisted in the form of floating polymorphisms [White (95); White & Morley (101)].

P. terminalis is by far the most intriguing species. All 192 males examined proved to be heterozygous for the "A" metacentric, whereas all 20 females were homozygous. That the A trivalent cannot consist of X, Y₁, and Y₂ chromosomes is proved by its concurrence with the highly characteristic XY pair. It may be a case of selective fertilization, or the missing recombinants may be zygotically inviable.

Piza's (55) equally remarkable cricket, *Eneoptera surinamensis* Saussure, in which regular segregation of an XY₁Y₂ trivalent occurs despite the absence of chiasmata within it, has been reinvestigated by Claus (15). Although he found an X₁X₁X₂X₂(♀):X₁X₂Y(♂) system, the puzzle regarding their method of segregation remains unsolved.

It is, at the moment, not possible to pass cytological judgment on the conspecificity of *P. approximatus* and *canadensis*, as has been so thoroughly done for the members of the *pusilla* complex in the acridid genus *Austroicetes* by White & Key (100). Doubtless, were it not for the parallel variation revealed in *terminalis*, the matching karyotypes and similar genetic systems of *approximatus* and *canadensis* would weigh heavily in favour of merging them.

Studies of fusion-fission polymorphism seldom provide unequivocal evidence of the direction of evolutionary change. Admittedly *Drosophila* and the Acrididae leave little room for doubt that reduction in number has prevailed, but White (96) and Wahrman (89) have presented strong claims for the reverse in other Orthoptera. In the Chillocorini, the evidence is unassailable that present-day downward changes in *Chillocorus* were preceded by increases, whereas in the co-tribal genus *Exochomus* the concurrent trend was downward. It is thus obvious that fusion, by restricting recombination, is as beneficial at one time as fission, through the release it provides [White & Chinnick (99)], is at another.

Paracentric inversions do not impair fertility in *Drosophila* and related forms because of the absence of crossing-over in the male and the elimination of dicentric bridges and acentric fragments via the polar bodies in the female. In order to persist in the population, the heterozygous inversion must therefore at least compensate for its occasional loss on the female side. Consequently, in organisms such as chironomids, in which chiasmata do occur in the male, it has usually been assumed that the inverted regions, which are commonly present, must be exempt from crossing-over. In actual fact, inversion bridges were found in *Chironomus tentans* males but not in *Glyptotendipes barbipes* (Staeger) [Basrur (3)]. In *tentans*, however, the 50 per cent sterility expected of a female after mating with a male heterozygous for a long inversion was by no means even approached. This, Beermann (7) showed, was because spermatids tied together by inversion bridges give

rise to nonfunctional double sperms. In *G. barbipes* it must be that the inversions are perpetuated because they prevent chiasmata from being formed within them.

A fundamentally similar but causally different process appears to operate in *Mantis religiosa* Linnaeus to maintain numerical constancy following non-disjunction of malorientated XXY trivalents [Callan & Jacobs (13)]. Moreover, when bivalent formation fails, reproductive wastage is further restricted by the resulting univalents "blocking" spermatocytes at metaphase I, so that aneuploid sperms are not produced. They suggest that this accounts for the absence of supernumerary chromosomes in mantids.

In view of the heterosis associated with inversion heterozygosity in natural populations of *Drosophila*, it comes as a surprise that Frizzi & Kitzmiller (20) find the expected heterozygotes totally absent from Palaearctic but not Nearctic members of the *Anopheles maculipennis* complex. It is not known whether this heterosis exists in *Anopheles*, but it is evident from the present-day range of fixed rearrangements in both groups that *Anopheles* has evolved through a state of balanced chromosomal polymorphism like that currently visible in *Drosophila*.

Wright (105) concluded that the reduced fertility imposed on translocation heterozygotes by nondisjunction must militate against their establishment, except under very special circumstances. How some of these adverse effects are, however, avoided has been shown through a study of isolated, colliery-inhabiting *Periplaneta americana* [Lewis & John (42)]. All individuals examined proved to be heterozygous for from one to three autosomal interchanges resulting from breakage and crisscross reunion at or near the centromere. They found multiple association essentially complete and disjunctive orientation almost perfect, the former because of near symmetry of the multivalents, the latter because, in addition, associations between the components were usually terminalized by the premetaphase stretch. The formation of aneuploid spermatozoa is thereby held to a minimum and the interchange hybrids are consequently fertile. That their establishment was favoured by the imposed change from outbreeding to inbreeding is reasoned by analogy with comparable studies of plants (38). A similar situation in *Blaberus discoidalis* (Serville), also pre-adapted for interchange, was described later [John & Lewis (39)].

Although space does not permit a detailed review of supernumerary chromosomes, the fifth class of chromosomal polymorphism, it is of interest to note that Seiler (70) has reported on a "supernumerary Y" in *Solenobia triquetrella* (Fischer) that is largely eliminated through the spermatozoa but retained through differential segregation into the female pronucleus.

MEIOSIS IN HYBRIDS

The wingless Australian grasshopper *Moraba scurra* Rehn consists of two races, an eastern one with $2n \sigma = 15$ and a western one with $2n \sigma = 17$ [White (95, 99)]. Spermatogenesis in hybrids establishes the equivalence of

the arms of the large metacentric (AB) in the east and the two acrocentrics (A & B) in the west [White (96)]. Meiosis was almost completely regular, but the occasional formation of quinquevalents including the A-AB-B trivalent is taken by White as critical evidence that the 17-race was derived, not by simple fragmentation of the AB, but through translocation involving one of the other chromosomes in the multivalent. This interpretation, of course, circumvents the difficulties encountered in theories of simple fragmentation, whereby an extra centromere and two extra telomeres need be acquired [White (97)].

The occurrence of the quinquevalents and other anomalies, indicative of homology of the end segments of several chromosomes, led White to question the validity of attributing, *ad hoc*, multiple associations in hybrids to interchange heterozygosity. He is inclined to regard the hybrids as translocation homozygotes rather than heterozygotes. As with Helwig's (28) interspecific and "intergeneric" grasshopper hybrids, White's studies revealed no evidence for paracentric inversions.

In western North America, *Chilocorus stigma* is replaced by four neo-XY species, two narrowly sympatric and homozygous for two fusions, and two allopatric and homozygous for three and six autosomal fusions [Smith (74)]. The last, *C. hexacyclus*, is the end product of a fusion process equivalent to that currently evident in *C. stigma*, just as *Philocleon anomalus* Roberts (27), homozygous for five autosomal and one autosomal-X fusion, culminates that in the Acrididae. As in other Chilocerini, chiasmata are restricted to one per euchromatic arm-pair: thus each species has 13 per cell, the number of ring bivalents being determined by the number of fusions.

Attempts to hybridize the sympatric species failed [Smith (74)], but one, *Chilocorus orbus* Casey, produced progeny with both *hexacyclus* and *tricyclus*, as did these between themselves. Highly consistent pairing in the F₁ males establishes the complete homology of the various fusions, there being one, three, or four trivalents and rarely fewer than 13 chiasmata per cell. Trivalent malorientation was variable and unexpectedly high. White's (96) *Moraba* hybrids showed almost no malorientation, comparable to that observed in eastern *C. stigma*, in grylloid natural heterozygotes [Ueshima (87)], and in XXY mantids in which "correct" alignment is usually achieved. The situation in *Chilocorus* hybrids presumably reflects the synthetic origin of their trivalents; they have not been subjected to adjustment by selection for near perfect co-orientation of centromeres.

Hybrids between *stigma* polymorphs and *orbus*, *tricyclus*, and *hexacyclus* establish the homology status of the various fusions. Semihomologous associations are always chains of four, never rings such as are found in *Periplaneta*. Extensive asynapsis and malorientation suffice to account for the high sterility observed. Inversion bridges were apparently absent, but associations comparable to the reduplication anomalies in *Moraba scurra* [White (96)] were present. Smith also recorded in *Chilocorus* one of the extremely rare cases of natural interspecific hybridization known in ani-

mals; details (unpublished) are essentially as outlined above. Natural hybrids of *Chironomus* were studied by Acton (2).

PARTHENOGENESIS

Hymenopteran males, being produced from unfertilized eggs, have gonial cells containing only one-half the number of chromosomes found in those of females, and the two sexes are accordingly generally conceded to be haploid and diploid respectively. Consequently, for a long time it was considered difficult to fit the Hymenoptera into any scheme based on a balance between male and female sex-determining genes. The first light was shed on the problem by Whiting's (102) demonstration that femaleness in *Habrobracon* depends on heterozygosity and maleness on hemizygosity for certain sex alleles, as revealed by the maleness of rare diploids (homozygous) that appeared following close inbreeding. Support from work on *Apis mellifera* Linnaeus failed to materialize until 1956, when, by the combined use of marker genes and artificial insemination, Rothenbuhler (60) presented strong evidence for the multiple sex-allele theory. Two recessive, nonlinked eye-colour genes introduced in sperm from genetically different males established that accessory sperm can develop in the production of gynandromorphs. When the males and females were closely related, moreover, spots of eye tissue were sometimes developed that can be explained only on the *Habrobracon* scheme. It is reasoned that diploid males are, as expected by Mackensen (44), inviable but that small pieces of biparental male tissue can survive in mosaic association with ordinary haploid male tissue. Still unexplained is the case of certain parasitic wasps in which diploid males are unknown, although brother-sister mating is the rule.

Although haplodiploidy of the hymenopteran germ tract is generally accepted, the fact that somatic cells are frequently polyploid has raised the question whether the same relative numerical proportions exist in them. Mirriam & Ris (48) and Risler (59) could find no such relationship in the honey bee [nor could O'Brien (51) in a haplodiploid coccid]. Furthermore, because of the mounting evidence that exceptional haploid cells, tissues, and organisms are elsewhere inviable, speculation has been aroused as to the actual chromosomal constitution of the hymenopteran male. Aware of this, Maxwell (47) has recently resurrected the obsolete theory of diplotetraploidy. She believes that the chromosomes in male sawflies are paired, both physically and morphologically, and that where the counts are odd-numbered the male is XO, and where they are even-numbered the male is XY. In confirming the genetic evidence for diploid males in the chalcidoid wasp *Nasonia vitripennis* (Walker), Pennypacker (54) likewise found some evidence of pairing in both haploids and diploids: her figures are, however, not particularly convincing. Whatever the true nature of these apparent or transient associations, they add little weight to theoretical arguments against haplodiploidy. On the other hand, although extensive genetic tests of higher Hymenoptera invariably demonstrate that males and females breed like

haploids and diploids, no planned experiments have yet been conducted in the lower Hymenoptera to supplement the cytological findings.

Much interest was aroused in 1941 by Smith's (71) report that *Diprion similis* (Hartig) gave counts of 14 and 28 instead of the 7 and 14 found in related species. Reinvestigating this species, Maxwell (47) concluded that Smith's determinations were erroneous, the true counts being 7 and 14.³

Unlike those of the Hymenoptera, haploid males in the diaspidine scales develop from fertilized eggs. By the use of radiation-broken "male" chromosomes, Brown & Bennett (12) have recently proved that haploidy is achieved in *Pseudaulacaspis pentagona* (Targioni) by the elimination of the paternal set late in cleavage. As Brown (10) points out, these findings confirm Schrader & Hughes-Schrader's (67) hypothesis that the heterochromatization during embryogeny of one set in male lecanoids could provide the basis for the evolution of true male haploidy. The coccids are noteworthy for the bizarre nature of their meiotic mechanisms, the interrelationships of which are currently being elucidated by Brown (9, 11).

Parthenogenesis is well known in the lower Diptera and occurs exceptionally in higher Diptera such as *Drosophila parthenogenetica* Stalker. Recently, regular diploid thelytoky has been described in the brachyceran *Lonchoptera dubia* Curran (Stalker (78)) and, for the first time in *Drosophila*, in a strain of *Drosophila mangabeirai* Malogolowkin [Carson, Wheeler & Heed (14)]. Triploid thelytoky has been discovered in *Chamaemyia polystigma* (Meigen) (Chamaemyiidae) by Stalker (79) and in *Cnephia mutata* (Malloch) (Simuliidae) by Basrur & Rothfels (4).

There is no precise cytological evidence as to how diploidy is maintained in the absence of syngamy in any higher Diptera. In *D. parthenogenetica* the genetical fact of recombination favours automixis [Stalker (77)]. As for the automictic *L. dubia*, its permanent structural hybridity suggests that the diploid number is reconstituted by the fusion of the inner two among the four products of a normal meiosis. This interpretation requires that chiasma formation be largely distal to the inverted region and attributes the 25 per cent sterility observed to chiasmata proximal to or within the inversions. A similar mechanism may operate in *D. mangabeirai* to account for its permanent heterozygosity, but certain relevant aspects of its cytogenetic system argue in favour of apomixis. Stalker (79) and Basrur & Rothfels (4) consider various means by which triploidy could be maintained in *C. polystigma* and *C. mutata*.

Although the spider beetles *Plinus latro* Fabricius and *P. hirtellus* Sturm have been recognized as separate, sexually dimorphic species since 1837, Moore, Woodroffe & Sanderson (49) have presented convincing evidence that *latro* is purely parthenogenetic. However, the type of parthenogenesis

³ It has since been ascertained (S. G. Smith, unpublished data) that Maxwell herself is in error. *D. similis* has 14 and 28 chromosomes, apparently arisen through centric fission. It is remotely conceivable that Maxwell's determinations were made on the sympatric species *Diprion frutetorum* Fabricius ($\sigma^7 = 7$, $\varnothing = 14$).

is unique among beetles in that *latro* females produce progeny, always females, only after mating with *hirtellus* males [or with *Ptinus pusillus* Sturm males, see Woodroffe (104)]. The *hirtellus* male and female are diploid and have normal meiosis, whereas the *latro* females are ameiotic triploids [Sanderson (63)]. It is not clear what role the spermatozoa play, but the egg can be partially activated by artificial means [Sanderson & Jacob (64)]. It would thus seem that, as in pseudogametic females of the psychid moth *Luffia lapidella* (Goeze) [Narbel-Hofstetter (50)], penetration by the spermatozoa simply acts to trigger the egg into further development. Sanderson (63) contends that it is logical to regard *latro* and *hirtellus* as a single polymorphic species; but, from the point of view of the integrity of their gene pools, both *latro* and parthenogenetic *lapidella* are clearly well along the road to becoming distinct taxonomic entities.

With the exception of *Micromalthus debilis* Leconte, *Ptinus latro*, and the chrysomelid *Adoxus obscurus* (Linnaeus), a bisexual diploid in North America, and a parthenogenetic triploid in Europe [Suomalainen (83)], all parthenogenetic beetles known cytologically are weevils. It is found that where a species comprises a number of races differing in degree of ploidy, they have dissimilar altitudinal or latitudinal distributions. As da Cunha (17) has stated, the steady accumulation of gene mutations and the elimination of gene exchange endow a purely parthenogenetic species with a high capacity to exploit a great variety of ecological niches.

Omitting *Liophloeus tessulatus* (Müller) [Suomalainen (82)], all the weevils studied by Suomalainen belong in the Otiorrhynchinae (in the North American sense). The 90 or so Japanese species studied by Takenouchi (84, 85), on the other hand, are broadly representative of the Curculionidae and include only three that are parthenogenetic—they are also polyploids. The 30 or so Canadian species examined by Smith (72; unpublished data) fall almost exclusively outside the Otiorrhynchinae: no parthenogenetic ones were encountered.

Takenouchi's (84) parthenogenetic *Listroderes costirostris* Schönherr has also been studied by Sanderson (62) in Scotland. Takenouchi found the oögonial number to be 33 and comments that "this corresponds to the triploid number common to parthenogenetic weevils." Sanderson, however, counted only 30 chromosomes, and entertained the idea of the species as a parthenogenetic diploid. The chromosome numbers of polyploid races do not necessarily form exact multiples of the basic number found in related bisexual species [Suomalainen (82)]. Since the latter have not been studied in *Listroderes*, no judgment can at present be reached as to the polyploid status of *costirostris*.

PSEUDOPOLYPLOIDY AND DNA

The compelling evidence that DNA is intimately associated with the gene, taken together with the general constancy of the chromosome complement of an organism, prompted Hughes-Schrader and Schrader to investi-

gate the possibility of using cytophotometric measurements of the average nuclear content of DNA ("DNA value") as an added cytotaxonomic tool in evaluating evolutionary relationships, especially where polyploidy is suspected.

Initial tests showed that neither the phenotype nor the karyotype in various species of the mantid genus *Liturgousa* is closely correlated with DNA quantity [Hughes-Schrader (31, 32)]. Since the values established for 14- and 27- chromosome species of the pentatomid *Thyanta* were closely similar, Schrader & Hughes-Schrader (68) put forward an interpretation of "longitudinal fragmentation" or "chromatid autonomy" as an alternative to polyploidy, whereby the chromosomes of the lower-numbered species are permanently dissociated into their component chromatids in the higher-numbered species. To accommodate *Arvelius albopunctatus* (De Geer), which also has 14 chromosomes but a DNA content twice that of the species of the co-tribal genus *Thyanta*, they suggested that each *Arvelius* chromosome is composed of twice as many fibrillae as are those of the 14-chromosome *Thyanta* species. This suggestion received support, in that 11 species of Pentatomini (mostly $2n=14$) fell into three DNA groups, thus indicating to these authors (35) that evolution in *Thyanta* had involved reduction in polyteny to one-half.

An additional opportunity of testing this theory was provided by the discovery of the apparently tetraploid *Pseudaulacaspis pentagona*. This species has $2n=8$ and 16 in males and females, exactly double the numbers found in all other diaspidine scales. Surprisingly, its DNA value proved to be one-half that in the haplodiploid species measured, leading to the conclusion that two downward shifts in valency had been associated with the doubling of the chromosome number [Hughes-Schrader (33)]. On the other hand, in species of the pentatomid *Banasa*, a similar increase in number has been accomplished without a significant change in DNA value [Schrader & Hughes-Schrader (69)]. If the Schraders' theory proves correct, it is probable that chromatid dissociation will be found the basis of the "polyploid" series in such Lepidoptera as *Leptidea* and *Erebia*, but supplementary changes have obviously been responsible for the several intermediate numbers.

Three species in the mantid genus *Ameles* are polymorphic for metacentric vs. acrocentric chromosomes [Wahrman (89, 90)]. Although the diploid numbers range from 18 to 29, the number of arms remains constant at 30 in accord with Robertson's "law." Wahrman & O'Brien (91), using Feulgen cytophotometry, have been able to confirm the cytological evidence for the evolutionary equivalence of the karyotypes tested.

Were it not for its polymorphs, monomorphic *C. stigma* might well be taken to be a tetraploid derivative of *C. hexacyclus*, especially in view of its sex-determining mechanism, for an XXY system is commonly regarded as symptomatic of polyploidy [see Goldschmidt (21)]. Nor presumably would DNA determinations provide convincing evidence to the contrary

for, while 26 euchromatic arms are present in both, *hexacyclus* female has only two heterochromatic arms, whereas *stigma* female has 26. This is clearly one answer to the question posed by Hughes-Schrader (34): "If the ancestral mantid karyotype has a relatively high number and DNA content . . . how could DNA content and total chromosome mass be halved . . . without loss of viability?"

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CHROMOSOMAL VARIATION AND ADAPTATION IN INSECTS¹

BY A. BRITO DA CUNHA

*Departamento de Biologia Geral, Faculdade de Filosofia, Ciências e Letras,
Universidade de São Paulo, São Paulo, Brasil*

INTRODUCTION

Organic evolution produces adaptation and biological progress. However, while adaptation is an ordinary result of the evolutionary processes, progress occurs less often. Characterization of biological progress is a difficult problem which for a long time has been the object of much discussion. Excellent analyses of the problems involved in biological adaptation and progress have been made recently by Huxley (64, 65), Simpson (104), and Thoday (125, 126). Thoday has the merit not only of defining clearly what biological progress is but also of giving scientific bases which permit evaluation of progress in evolution. He defines biological progress as increase in fitness for survival. He defines as fit those organisms which are well adapted to their existing environments and whose descendants will fit future environments. He also says that "environment change and ability to accommodate to that change are therefore the keys to understanding the nature of fitness and progress." According to Thoday's definition, the biological mechanisms which improve the adaptation of the organisms to their present environments and which increase the probability of perpetuation of their kind by reproduction through environment changes are progressive.

The environment is never uniform in space and never constant in time. Evolution maintains the organisms adapted to the environment, but it also changes the biological environment. Evolution, in general, produces adaptation, but it is progressive only if the changes occurring in the organisms increase the probability of survival. To survive genetically the organism must be adapted to the environment and be able to leave offspring:

The probability of survival will depend upon adaptation, the genetical stability of the population (its capacity to remain adapted), the variability of the population (its capacity to change) and the stability of the environment (which determines its need to change). Increase of any of these, provided it does not involve corresponding decrease of another, will be biological progress [Thoday (126)].

Evolutionary progress depends on a successful equilibrium between two antagonistic factors of the population: genetical stability and genetical variability. Both are determined by the genetic systems. The variability of populations is dependent also on the capacity of the individuals which compose them to accommodate themselves to the environmental variants; i.e., it depends on the versatility of the individuals. Thoday (126) summarizes:

¹ The survey of literature pertaining to this review was concluded in December, 1958.

Biological progress may be in principle brought about by any evolutionary change that so improves the genetical systems that the antagonism between genetical stability and genetical versatility is reduced, any change that increases individual versatility, any change that increases the diversity of types and hence the number of environments to which they are adapted forms, and any change that permits the organism to control its environment in such way that the environment remains suitable or becomes more suitable for the organism.

Genetical knowledge for a few groups of insects is already sufficiently advanced to permit a study of the genetics of their adaptations and of their evolutionary potentialities for progress. Studies of the chromosomal polymorphism of insect populations give the best bases for such a study. Data bearing on most of the evolutionary factors mentioned above have been obtained in the cytogenetical studies of several insect populations. We propose to analyze here the relations between chromosomal polymorphism of insect populations and their evolutionary adaptation and progress.

Chromosomal polymorphism is well known in natural populations of Diptera [da Cunha (21)], Orthoptera [White (137)], and Coleoptera [Smith (108)]. That it is little known in other groups probably results more from absence of population research than from the nature of the organisms.

The commonest forms of chromosomal variants found in the natural populations of Diptera are the paracentric inversions; while in the Orthoptera and Coleoptera the usual variants are pericentric inversions and chromosomal fusions, respectively. In both Orthoptera and Coleoptera, supernumerary chromosomes are relatively frequent, and they are known also in Homoptera, Heteroptera, Neuroptera, and Diptera.

Chromosomal inversions are well known in their effects in the genetic systems [White (137); Darlington (30)] and their evolutionary roles will be our main concern in this review.

Despite the fact that supernumerary chromosomes are rather common in animals and in plants, their role in evolution is unknown. Although it is in a few cases recognized that they have selective effects, their genetics is very little understood; for this reason they will not be discussed in this paper.

CHROMOSOMAL POLYMORPHISM AND INDIVIDUAL ADAPTABILITY

Chromosomal inversions prevent the production of chromosomal recombinations by crossing-over within the inverted sections. When crossing-over occurs inside heterozygous inverted section, the new chromatids produced are eliminated, as shown by Stone & Thomas (122), Beadle & Sturtevant (5), Sturtevant & Beadle (124) and Carson (11). The genic combinations organized inside the inverted sections of the chromosomes remain, therefore, intact in the heterozygotes. Crossing-over in heterozygotes for paracentric inversion produces nonviable chromatids with either two centromeres or none and with deficiencies and duplications. However, in organisms like *Drosophila*, where crossing-over does not occur in the males, paracentric inversions do not decrease fertility. The production of a chromatid bridge,

a result of the chromatid with two centromeres, in the ovogenesis meiosis insures that the egg receives only a normal, nonrecombinant chromatid, as postulated by Beadle & Sturtevant (5) and proved by Carson (11) in *Sciara impatiens* Johannsen. Spermatozooids with abnormal chromosomes are produced in the males heterozygotes for paracentric inversions in organisms where crossing-over occurs in males. However, no decrease in fertility and fecundity occurs in those males, as found by Acton (1, 2, 3) in *Chironomus*. The reason that fecundity of males heterozygous for paracentric inversions and with crossing-over is not decreased was discovered by Wolf (142) in *Dicranomyia trinitata* (Meigen) and by Beermann (6) in *Chironomus tentans* Fabricius. Crossing-over within the heterozygous inverted section in the males of those species produces chromatid bridges which attach themselves to the spermatids. The attached spermatids produce double spermatozooids which are incapable of fertilization. The decrease of the number of normal spermatozooids is too small to affect the fertility of the males heterozygous for the paracentric inversion. Paracentric inversions are therefore cytologically neutral in insects like *Drosophilidae*, *Sciaridae*, *Cecidomyiidae*, *Bibionidae*, and *Agromyzidae*, where no crossing-over occurs in the males. In insects like *Chironomidae*, *Culicidae*, *Limoniidae*, and *Ptychopteridae*, where crossing-over occurs in males, the paracentric inversions are sometimes neutral and detectable decrease of fertility is not produced.

Pericentric inversions are always adverse where crossing-over occurs. Crossing-over inside the loop of heterozygous pericentric inversions invariably produces anomalous chromatids and no chromatid bridge. The absence of bridge does not insure the permanence of the normal chromatids in the egg cell and consequently sterility occurs. Sterility from pericentric inversion occurs only in the females when crossing-over does not occur in the males. Despite the cytologically deleterious effects of pericentric inversion, it is known to occur in natural populations of *Drosophila robusta* Sturtevant [Carson (16)], *Drosophila algonquim* Sturtevant & Dobzhansky [Miller (95)] and *Drosophila ananassae* Doleschall [Freire-Maia (57)]. In *D. robusta*, one of the two recorded pericentric inversions, 3 L-R, is fully viable and widespread in North-Central United States, where it reaches a frequency as high as 50 per cent in some localities. The other pericentric inversion, 2 L-R, was found only once in nature but laboratory results indicate that it is also heterotic, since it was maintained in stock for more than two years in heterozygous condition [Carson (16)]. As pointed out by Acton (1, 2, 3), the genetical advantages of pericentric inversions can be higher than their deleterious effects and the inversion will then survive in the population. The pericentric inversions can also become neutral if by selection chiasmata are suppressed within the inverted section. However, when a paracentric inversion with approximately the same genetic contents of the pericentric inversion occurs, the pericentric is eliminated by natural selection in favor of the advantages of the paracentric. This is probably one of the reasons for the rarity of pericentric inversions. The common occurrence

of pericentric inversions in Orthoptera results from the suppression of chiasmata in the inverted section [White (137)].

Translocations are generally deleterious cytologically. However, natural selection may eliminate their adverse effects by special mechanisms, as in *Periplaneta americana* (Linnaeus) [John & Lewis (66, 67, 87)] where complete terminalization and a premetaphase stretch insure regular disjunction. Translocations have been found in populations of *D. ananassae* [Dobzhansky & Dreyfus (40); Freire-Maia (58)], but their behavior at meiosis and their role in the populations are unknown.

The chromosomal variants or morphs are therefore cytologically neutral or harmful. When deleterious, mechanisms like chiasma suppression, complete terminalization, premetaphase stretch, and spermatozooids fusion evolve by natural selection and decrease the adverse effects.

The individuals heterozygous for chromosomal inversions have an adaptive value higher than those of the homozygotes in most of the cases studied. This higher adaptive value of heterozygotes is attributable not to the inversions themselves but to their genetic content. This fact was well shown by Dobzhansky (35). Heterozygotes for two different genic sequences are heterotic when the two chromosomes have the same geographic origin but are not heterotic when they come from different regions. To be heterotic the genic contents of the inversions must be co-adapted by selection.

Mainly as a result of research in *Drosophila*, the physiological aspects of the adaptive differences between individuals heterozygous and homozygous for inversions are becoming better known. In all examples observed so far, if response to several environmental factors and many physiological and biological characteristics were studied, the heterozygotes revealed an adaptive value higher than the homozygotes. Homozygotes may be superior to heterozygotes in a few characteristics but, in the mean, they are inferior.

Wright & Dobzhansky (143) have found that in *Drosophila pseudoobscura* Frolova inversion heterozygotes were superior to the homozygotes in adaptive values at 25°C. but similar to them at 16°C. Spiess (110) obtained similar results with *Drosophila persimilis* Dobzhansky, but in this species the superiority of the heterozygotes is manifested at 16°C. but not at 25°C. It is interesting to notice that *D. persimilis* live at higher altitudes than *D. pseudoobscura* and hence should be more adapted to lower temperatures than *D. pseudoobscura*.

Inversion heterozygotes are superior to homozygotes but their relative adaptive values change with seasons, altitude, food, and other factors. Altitudinal gradients in the frequencies of inversions have been found by Dobzhansky in *D. pseudoobscura* (32, 33) and in *D. persimilis* (33) and by Stalker & Carson (117) in *D. robusta*. Seasonal changes in the frequencies of the chromosomal types have been disclosed by Dobzhansky (33, 39) in *D. pseudoobscura*. The population remains polymorphic because of the fact that through all the changes the adaptive value of the heterozygotes remains higher than the value of the homozygotes. Dobzhansky & Levene

(41) obtained direct proof that in nature there is differential mortality in favor of heterozygotes by comparing egg and adult samples taken from nature. The egg samples were obtained from captured females whose eggs were raised in optimal conditions. The analysis of the larval chromosomes showed heterozygotes and homozygotes to be present with the frequencies expected by the Hardy-Weinberg law. The adult sample was made by capturing males, crossing them with females of known chromosomal constitution, and analyzing cytologically the F_1 . The F_1 analysis gives the cytological constitution of the adult males. This analysis showed that among the adults the heterozygotes are in significant excess over the homozygotes. Epling *et al.* (53) made a similar analysis and found no excess of heterozygotes. Differential mortality may or may not exist, depending, of course, on the gene contents of the chromosomal variants and on the environment. Differential mortality in favor of inversion heterozygotes was found in laboratory populations of *D. robusta* [Levitan (76)] and of *Drosophila subobscura* Collin [Kunze-Mühl, Müller & Sperlich (70)], but it was not found in their natural populations where the chromosomal genotypes appear in the proportions expected by the Hardy-Weinberg law. Acton (2, 3) found no differential mortality in chromosomally polymorphic natural populations of both *C. tentans* and *Chironomus dorsalis* Meigen. Differential mortality in favor of heterozygotes for inversions was also found by White (138, 139) in the natural populations of the Australian grasshopper *Moraba scurra* Rehn.

The situation regarding seasonal fluctuations in the frequencies of chromosomal types is remarkable in *D. robusta*. Carson & Stalker (17) and Carson (16) found no seasonal changes in a St. Louis country population. Identical results were obtained by Levitan (76) in a New Jersey population. However, Levitan (77, 78, 82) found very clear seasonal fluctuations in a Blacksburg, Virginia, population. Two gene arrangements—2 L-3 and XL-1—are more frequent in wintering flies and decline with spring and summer. In addition, this change is more pronounced in females than in males. These two gene arrangements are clearly connected with cold, since they are more abundant also in northern and high-altitude populations.

Da Cunha (19) and Dobzhansky & Spassky (45) studied the effects of different types of food on the adaptive values of chromosomal heterozygotes and homozygotes. Again, their relative adaptive values change in accord with the environment—in this case food. The heterozygotes are superior with most of the foods.

Studies of the physiological properties of different gene arrangements have been performed mainly by Spiess and co-workers in *D. persimilis* and by Moos and Heuts in *D. pseudoobscura*.

Spiess, Ketchel & Kinne (114) found that the heterozygotes WT/KL are superior to both homozygotes WT/WT and KL/KL in fecundity and longevity. The WT/WT flies maintain a daily egg production of 13 to 15 eggs for about 100 days at 15°C. The KL/KL flies start laying about 13 eggs per day but the production falls to 8 to 9 after 10 days. The heterozygous

WT/KL flies start laying about 20 eggs per day and this higher fecundity is maintained for approximately 60 days, dropping then to the level of productivity of the WT/WT flies. This productivity of the WT/KL flies, higher than that of the homozygotes, gives to the heterozygotes not only a probability of larger offspring but also an advantage in competition with the KL/KL and WT/WT flies. The longevities of the three types at 15°C. were found to be: WT/KL = 174 days, WT/WT = 166 days, and KL/KL = 165 days.

Spiess, Terrile & Blumenheim (116) measured the wing dimensions as well as their beats in WT/WT, WT/KL, and KL/KL flies. Differences were found but no evidence for heterosis. The egg-laying capacities of ST/WT, ST/ST, and WT/WT flies were studied by Spiess (111). In laboratory populations the adaptive values of the three types are: ST/WT = 1.0, WT/WT = 0.9, and ST/ST = 0.634. The heterozygotes are therefore neatly superior in adaptive values, which are measurements of the performances of the genotypes in respect to all the biologically important characteristics. However the same is not true for the adaptive value component studied, namely, fecundity. The ST/ST flies were found to be equal or slightly superior to WT/WT. The heterozygotes derived from WT/WT mothers had an egg-laying capacity equal to that of WT/WT flies, but those derived from ST/ST mothers had a production about one-half that. This is a clear case of maternal influence and shows that the heterozygotes may be inferior to the homozygotes in some characteristics, despite their superior adaptive value.

Spiess & Schuellein (115) made a detailed analysis of several characteristics of flies WT/WT, WT/KL, and KL/KL. They analyzed the survival of females, their egg-laying capacities, the rates of development, and the number of adults eclosing from 100 eggs. They found the heterozygotes WT/KL to be superior in all those characters. On the basis of the flies' performances, they estimated the adaptive values to be: WT/KL = 1.00, WT/WT = 0.811, and KL/KL = 0.643. A further work [Spiess (113)] presents results of an analysis of the egg-laying capacities under optimal and under crowded conditions, of the survival of preadults, of the rate of development and the rate of maturation of the WT/WT, WT/KL, and KL/KL individuals. The heterozygotes were found to be superior in egg-laying capacity and in survival of preadult stages; they were similar to the homozygotes in rate of development and of maturation.

A very important finding obtained by Spiess (113) and by Spiess & Schuellein (115) was that the heterozygotes were always more homeostatic than the homozygotes.

Wallace (131) analyzed physiological properties of different chromosomal genotypes in *D. pseudoobscura*. The gene arrangements studied were SR and ST in the X chromosome. The SR/ST females were found to be superior to the homozygotes SR/SR and ST/ST in all characteristics studied, namely in larval competition, longevity, fecundity, and egg hatchability. The results obtained in this analysis permitted calculation of the relative adaptive values

of the three chromosomal genotypes. The adaptive values are: at 25°C.—SR/ST=1, SR/SR=.021 to .014, ST/ST=.314 to .277; at 16°C.—SR/ST=1, SR/SR=.343 to .278, ST/ST=.849 to .734.

Heuts (61, 62) observed (also in *D. pseudoobscura*) the viabilities of pupae and of adults, as well as the longevity of adults at different humidities and temperatures. The chromosomal combinations studied were all made with chromosomes from the same locality (Piñon Flats, California). He found that the heterozygotes ST/CH are intermediate between the homozygotes in longevity at high temperature, but superior at low temperature. He also found that when the pupae develop at a relative humidity of 70 to 75 per cent, the AR/AR adults live longer than ST/ST or CH/CH at any humidity. The larvae CH/CH were found to be inviable at 28 to 30°C., but at this temperature ST/ST survive better than ST/CH. Results of the same type were obtained by Levine (74) and by Spassky (109). Spassky found, studying AR, ST, and CH homozygotes, that CH/CH has a higher viability at high temperature and dry food and AR/AR at a lower temperature and humid food.

A detailed analysis of the physiological properties of different chromosomal types was made by Moos (96) in *D. pseudoobscura*. The chromosomal types selected were the homozygotes and heterozygotes for the third chromosome arrangements ST and CH from Piñon Flats, California. Moos found that at 25°C. the heterozygotes ST/CH were superior to the homozygotes ST/ST in the rate of development, in the longevity of males and females, equal or inferior in fecundity, and inferior in larval and pupal viabilities. The homozygotes CH/CH were inferior to ST/CH and to ST/ST in all the characteristics studied. Like Spiess (113) with *D. persimilis*, Moos found in *D. pseudoobscura* that the inversion heterozygotes ST/CH showed variances lower than those of the homozygotes.

Dubinin & Tiniakov (46 to 51) obtained in *Drosophila funebris* (Fabricius) good evidences that chromosomal inversions are adaptive in this species. They found differential survival among the different chromosomal types at low temperature. They also found, as will be shown below, correlation between inversions and ecological conditions. Philip *et al.* (98) observed clear indications of high adaptive value of inversions in *D. subobscura*. A strain heterozygous for three inversions kept all the inversions after 15 generations of inbreeding, showing the strong heterotic effect of the inversions. They found the homozygotes to be as viable as the heterozygotes in some conditions, but in other conditions the heterozygotes were the most viable and fertile types.

The most important data on the adaptive value of inversions have been provided by populations of *Drosophila* kept in laboratory. The populations are kept in special cages with conditions as constant as possible. The populations can be bred continuously for as long as necessary and samples taken periodically. Wright & Dobzhansky (143), using this technique, found that in populations of *D. pseudoobscura* with two gene arrangements derived from

populations of the same geographic locality the two chromosomal types are maintained by selection and an equilibrium is reached. At equilibrium the frequency of the two chromosomal types is always the same, regardless of the initial frequency, provided the experiments are made at 25°. For example, when the populations are made with individuals with the two chromosomal types AR and CH from Piñon Flats, California, the equilibrium is always obtained when the frequency of AR reaches 75 per cent and that of CH 25 per cent. When the experiment is started with a low frequency of AR, the latter increases gradually until it reaches 75 per cent. When the experiment is inverted, that is to say, is started with very high frequency of AR, this decreases to the same equilibrium point of 75 per cent. The changes in the frequencies of the gene arrangements from the initial composition to the equilibrium point can be used to calculate the adaptive values of the chromosome types. As an example, the following adaptive values were obtained by Dobzhansky (34) in populations with two alternative chromosomal types from Piñon Flats:

ST/AR=1	ST/ST=0.8	AR/AR=0.5
ST/CH=1	ST/ST=0.8	CH/CH=0.5
AR/CH=1	AR/AR=0.9	CH/CH=0.5

This equilibrium is reached and maintained because the adaptive value of heterozygotes is higher than that of the homozygotes. The equilibrium point is determined by the relative adaptive values of the three types.

A very important factor in determining the equilibrium point is the differential mortality between egg and adult stage, as found by Dobzhansky (32). While the chromosomal types are found in the frequencies expected by the Hardy-Weinberg law in the egg samples taken from the cages, in the adult samples there is always a significant excess of heterozygotes. However, differential mortality is only one of the components which determine the adaptive value. When the experiments are repeated at 16°C., no change occurs because at low temperature the adaptive values of the chromosomal types in *D. pseudoobscura* are similar. The adaptive values depend, therefore, on environmental factors like temperature.

Another environmental factor of selective importance in the populations studied was food. Da Cunha (19) maintained several populations receiving different yeasts. The gene arrangements were ST and CH obtained in Piñon Flats (California). The adaptive values of the chromosomal types in differently fed populations can be seen in Table I. As the table below shows, the adaptive values vary with the food used. Even more, with four diets, no heterosis was found. Similar results were obtained by Dobzhansky & Spassky (45).

Degree of crowding is still another factor important in the determination of the adaptive values. Birch (8) analyzed population cages of *D. pseudoobscura* containing flies with the gene arrangements ST and CH. The populations were kept in different degrees of crowding. In both crowded and un-

TABLE I

ESTIMATES OF THE ADAPTIVE VALUES OF THE CHROMOSOMAL TYPES
IN POPULATIONS RAISED WITH DIFFERENT FOODS

Food	Adaptive value		
	ST/ST	ST/CH	CH/CH
<i>Saccharomyces cerevisiae</i> Hansen	0.71	1	0.32
<i>Rhodotorula mucilaginosa</i> (Jørgensen) Harrison	0.83	1	0.49
<i>Candida parapsilosis</i> (Ashford) Langeron & Talice	0.89	1	0.54
<i>Zygosaccharomyces dobhzhanskii</i> Shihata	1.06	1	0.35
<i>Zygosaccharomyces drosophilae</i> Shihata	<0	1	<1
<i>Kloeckeraspora apiculatus</i> (Lindner) Dvornik	1.55	1	1.00
<i>Candida guilliermondii</i> (Castellani) Langeron & Guerra	0.80	1	0.32
<i>Candida krusei</i> (Castellani) Berkhout	<1	1	<1
Bacteria 3	1.14	1	0.37
Bacteria 9	1.15	1	0.57

crowded populations, the heterozygotes ST/CH are superior to the homozygotes. But, while the homozygotes CH/CH are superior to the ST/ST in uncrowded populations, the reverse is true in the crowded ones. These results suggest an explanation to what happens in nature. In the populations from which the chromosomes studied derived (Piñon Flats, California) CH increases in frequency in spring, when the population has small density, while ST increases in summer when the population density is much higher. Probably in nature as in the laboratory, the degree of crowding is one of the factors determining the changes in the genetic composition of the population.

Studying populations of *D. persimilis* maintained in population cages, Spiess (110) obtained results similar to those observed by Dobzhansky and Wright for *D. pseudoobscura*—with, however, one very important difference. In *D. persimilis* heterosis for inversion heterozygotes occurs at 16.5°C. but not at 25°C. The situation is the reverse of that found in *D. pseudoobscura*.

Levitan (76) found that all the inversions of *D. robusta* which he tested in population-cage experiments are heterotic. The experiments showed that the adaptive values of the different gene arrangements of *D. robusta* are easily changed by environmental factors. In *Drosophila willistoni* Sturtevant [da Cunha (20, 22, 23); Pavan, Dobzhansky & da Cunha (97)] chromosomal inversions are adaptive in nature as well as in the laboratory. In this species, however, the chromosomal types seem to be less sensitive to nutritional factors than in the other species. Da Cunha (22, 23) maintained in cages several experimental populations receiving six different yeasts as food. The populations were heterozygous for 18 different inversions. The evolution of the populations was the same with all the yeasts studied. It is interesting to

note that in *D. pseudoobscura*, when only two alternative gene arrangements were used, differences in their frequencies resulted with all the foods used; whereas in the experiments with *D. willistoni*, where the number of variants was much higher than in *D. pseudoobscura*, all the variants behaved in the same way regarding food.

The adaptive values of the chromosomal inversions are strongly influenced by other gene arrangements present in the population and even by their frequencies. The reciprocal influence of the different gene arrangements was well demonstrated by the experiments of Wright & Dobzhansky (143) and of Levene, Pavlovsky & Dobzhansky (72) with *D. pseudoobscura*. These workers prepared population cages with the gene arrangements ST, AR, and CH. For some populations only two gene arrangements were used and for others, three. The adaptive values of the several chromosomal types were calculated in the two-gene-arrangement and the three-gene-arrangement populations. The same genotype had different adaptive values in the two- and the three-inversion populations. For example, AR/AR is almost lethal in the three gene arrangement populations, while CH/CH has an adaptive value twice that of AR/AR. Nonetheless, in the populations in which only AR and CH are present, AR/AR is superior to CH/CH. In the triple experiment, the homozygotes ST/ST are superior to ST/CH, but in the double experiment the reverse is true.

White (139) has found a similar interaction of heterotic systems in the Australian grasshopper *M. scurra*. Two chromosomes of this species are variable, having heterotic inversions. When the populations are polymorphic for the gene sequences ST and B1 in the chromosome "CD" and for ST and Tid in "EF," an interaction occurs between them. As a result, the populations are deficient in B1/B1 and ST/ST individuals. Furthermore, individuals are deficient in which Tid occurs together with ST "CD," in homozygous as well as in heterozygous conditions. Spiess (112) observed a remarkable situation in *D. persimilis*. He found in laboratory populations that within a same population the adaptive value of a chromosomal genotype is not constant but varies according to the gene arrangement frequencies. When the gene arrangements KL and WT are together in a population, KL/KL is superior to WT/WT when the frequency of KL is above 55 per cent and inferior at lower frequencies of KL. The adaptive value of the chromosomal types are therefore dependent on the chromosomal types present and on their frequencies.

The heterozygosis for chromosomal inversions in the third chromosome of *D. pseudoobscura* produces an increase in the amount of recombination in the other chromosomes. Epling, Mitchell & Mattoni (54) concluded from their work that the recombinants produced by crossing-over in the inversion-free chromosomes have effects in the adaptive values of the inversions and thus influence their frequencies. The fluctuations in the frequencies of the chromosomal inversions are induced not only by environmental but also by genotypic changes. There are some long-range population changes which

may perhaps be explained as responses to such internal changes. Dobzhansky (31, 37) and Epling & Lower (52) reported remarkable variations in the populations of *D. pseudoobscura* in the Yosemite and in the San Jacinto regions of California. The gene arrangement PP was exceedingly rare in California before 1951. To be more specific, Epling found three PP chromosomes in July 1951 in a population in the San Jacinto region after the analysis of more than 22,000 chromosomes. By the next March (1952) in the same place the frequency of PP had reached 10.8 per cent. This very fast increase in the frequency of PP in the San Jacinto area was paralleled by identical changes at Mather in the Sierra Nevada region. In San Jacinto, the frequencies of four other previously rare gene arrangements had increased together with PP. As no environmental change was detected, the most probable explanation for this remarkable phenomenon is an adaptation of the populations to internal changes produced by the formation of new genotypes by recombination or by mutation.

When an organism is subject to changes in its environment, some of its physiological processes have to alter accordingly but others have to remain constant for the survival. The constancy of some characteristics through environmental change is made possible by the adaptation of others. The constancy of physiologically important processes through environmental changes was called homeostasis by Cannon. As pointed out by Cannon and also by Lewontin (88) and others, both lability and stability are characteristic of homeostasis. As Lewontin (88) says "A homeostatic organism maintains constant those aspects of its physiology whose constancy is necessary for survival. Conjugately, such an organism will be characterized by lability of those processes where lability is adaptive." Lewontin concludes: "A genotype is homeostatic if individuals of this genotype can so adjust their physiology and morphology as to survive and leave offspring in a wide variety of organisms."

There is already a large amount of evidence that in cross-fertilizing organisms the heterozygous genotypes are more homeostatic than the homozygous. For an excellent review of the subject to 1953, see Lerner's book *Genetic Homeostasis* (Oliver and Boyd, Edinburgh). Very important papers published more recently are those by Clarke & Maynard Smith (18), Dobzhansky & Levene (43), Hollingsworth & Maynard Smith (63), Lewontin (88, 89), J. and S. Maynard Smith (94), and Thoday (125, 126).

The higher adaptive values of the chromosomal inversion heterozygotes are generally correlated with better homeostasis. Heuts (62) got a first indication of the more perfect homeostasis of inversion heterozygotes in *D. pseudoobscura*. He studied the longevities at different temperatures of ST/CH, ST/ST, and CH/CH flies and observed that when the longevities of the same genotype at different temperatures are compared, the differences in mean longevities are lower for the heterozygotes. To illustrate, when the mean longevities at 28 to 30°C. and 0 to 4° were compared he found a difference of 10.68 days for ST/ST, of 7.58 for CH/CH, and of 6.21 for ST/CH.

The heterozygotes were, therefore, more constant in longevity in the temperature range studied. Spiess, Ketchel & Kinne (114) observed that the coefficient of variation of daily average egg production in *D. persimilis* is lower in inversion heterozygotes than in the homozygotes. These results were extended by Spiess & Schuellein (115), who found the inversion heterozygotes to present a lower coefficient of variation than the homozygotes in the number of eggs laid per day, in hatchability of the eggs, and in the eclosion of adults.

Moos (96), in her detailed analysis of the physiological properties of ST/ST, ST/CH and CH/CH *D. pseudoobscura*, obtained results showing clearly that the heterozygotes tend to have a variance lower than those of the homozygotes. Her results are shown in Table II.

The results reviewed in the table show that (a) the chromosomal inversion heterozygotes are superior to the homozygotes in most of the conditions studied and that (b) the chromosomal inversion heterozygotes have a more perfect homeostasis than the homozygotes. The chromosomal inversion heterozygotes are not only more adapted to the conditions studied but also more able to survive environmental changes. The chromosomal inversion heterozygotes are definitely the genotypes with higher survival probabilities in their populations.

CHROMOSOMAL POLYMORPHISM AND POPULATIONAL ADAPTABILITY

The genes present in a population constitute a well-integrated gene pool based in its organization mainly upon selection of heterozygotes. As it was well said by Wallace & Vetukhiv (133):

Various experimental techniques have revealed three levels of integration within the gene pools of local populations: (1) integration based upon epistatic interactions between homozygous loci, (2) coadaptation of different gene arrangements within local populations involving both heterosis and epistasis, and (3) the integration of entire gene pools through selection for heterozygosity.

The absence of recombination inside the inverted sequences in the chromosomal inversions permit a genic organization that would otherwise be impossible. As shown by Dobzhansky (35), the alternative gene sequences present in a population are coadapted to give heterosis and the coadaptation is developed by the action of natural selection. The development of coadaptation between different gene arrangements was observed and analyzed by Dobzhansky & Levene (42), Dobzhansky & Pavlovsky (44), and Levine (73) in *D. pseudoobscura*.

Dobzhansky said (36):

According with the balance hypothesis, the adaptive norm is an array of genotypes heterozygous for more or less numerous gene alleles, gene complexes and chromosomal structures. . . . If the fitness of homozygotes of many gene alleles and genes complexes is low, there will be a selection pressure favoring the development of series of multiple alleles at many loci and of a multiplicity of alternative gene complexes in most chromosomes. This process will lead to reduction of the incidence of genic and

TABLE II
SUMMARY OF VARIANCE DATA*

	Variance	
	CH/CH	ST/ST
<i>Longevity</i>		
16°C. ♀ ♀	1.56	6.06
16°C. ♂ ♂	2.01	3.76
25°C. ♂ ♂	0.36	1.17
25°C. ♀ ♀	0.51	0.85
16°C. ♀ ♀	1.90	1.94
25°C. ♀ ♀ †	23.49	5.06
<i>Fecundity 25°C. A</i>		
0 to 10 days	7.06	9.31
11 to 20 days	1.32	1.24
21 to 30 days	29.84	35.14
31 to 40 days	2.38	6.11
41 to 50 days	0.12	1.06
<i>Fecundity 25°C. B</i>		
0 to 10 days	2.57	2.71
11 to 20 days	2.64	2.55
21 to 30 days	2.43	1.43
31 to 40 days	2.08	0.86
41 to 50 days	0.29	1.23
<i>Fecundity 16°C.</i>	2.80	4.93
<i>Larval and Pupal Viability</i>		
A	1.36	3.35
B	1.12	1.48
C	5.32	1.95

* Variances are relative, that of ST/CH being arbitrarily set as 1.00.

† Fecundity data.

chromosomal homozygotes and to improvement of the adaptedness of the population as a whole. Once inaugurated this process may be self-propelling. The less frequent the homozygotes, the less important their fitness for the welfare of the populations as a whole.

While the entire gene pool is integrated, primarily by the selection of genes which give good combinations in most of the genotypes, the chromosomal inversions permit an organization of gene complexes protected against dissociation. The genotype integration can go even a degree further. Carson & Stalker (17), Levitan (79, 80, 81, 83, 84) and Levitan & Salzano (86)

observed that some independent inversions tend to occur together even when recombination between them should occur. The nonrandom association of the inversions results from the higher adaptive value given by them when together than when separated. Carson (12) and Levitan (83) showed that crossing-over between such linked inversions is much lower than should be expected on the basis of the distance between them. Levitan (84) analyzed the distribution of inversions in all the Diptera in which two or more inversions occur. His analysis reveals that association of gene arrangements in systems of overlapping, included, or independent inversions is much more common than could result from mere chance. Such nonrandom associations have been found in two species of *Chironomus* and in 18 of *Drosophila*. Levitan argues convincingly that the most probable mechanism for the maintenance of the nonrandom associations is natural selection acting against the recombinant arrangements.

A different type of integration in natural population is the absence of triads of inversions. Wallace (132) pointed out that if three overlapping inversions coexisted in a same population, serial transfer of genes between them could be produced by crossing-over and the coadaptation of the gene combinations would be disturbed. Wallace presented this hypothesis to explain the fact that in the populations of *D. pseudoobscura* only two members of such triads coexist. Because Wallace's hypothesis is not in agreement with the data obtained by Levitan, Carson & Stalker (85) in the analysis of the populations of *D. robusta*, it seems not to be a general explanation.

Acton (1, 2, 3) pointed out that the appearance in a population of an inversion similar to others already present may cause difficulties in their coadaptation and result in elimination of one of the sequences. This fact could explain why similar inversions within a population are rare, why inversions which are adaptive in the original population are not adaptive in others, and also why some inversions are endemic.

No one genotype, either homozygous or heterozygous, is likely to be a paragon of adaptability, superior to all other genotypes in all environments which the species is confronted with. The adaptability of Mendelian population may, then, be advanced if it contains a variety of genotypes suited to different adaptive niches and facies of the environment which the population inhabits [Dobzhansky (36)].

Two kinds of variabilities function in populations and permit adaptation to environmental diversity in space and in time. One type is the genic variability which is free for recombination. The other type is the genic variability organized in integrated and coadapted gene complexes protected against recombination by special chromosomal arrangements. The balance between adaptation and variability of the populations is created by the balance between free variability and variability tied in gene complexes.

The great advantage of heterotic gene complexes, as of chromosomal inversions, is that the population may change adaptively without losing the

variants. The seasonal fluctuations in *D. pseudoobscura* [Dobzhansky (32, 34, 37)] give a clear example of the advantages of heterotic variability. In the populations of *D. pseudoobscura*, the frequency of some gene arrangements common in spring decrease during summer and autumn, when other gene arrangements increase. The populations change with the seasons and are always adapted. However, variability is not lost with the changes because the heterozygotes are always superior to the homozygotes. We have, therefore, the ideal condition in which the population is always changing to fit the environment without any loss of variability.

The relative role of free and of tied variabilities in the adaptation of populations can be well seen in *D. willistoni* [da Cunha, Burla & Dobzhansky (25); da Cunha & Dobzhansky (26); da Cunha *et al.* (27); Dobzhansky (38); Townsend (127, 128)]. *Drosophila willistoni* is the most widespread Neotropical species in the genus. Its distribution area reaches from Florida to Argentina and within this very large area, *D. willistoni* is present in every habitat where *Drosophila* can be found. The chromosomal variability of *D. willistoni* is the largest known, 50 different inversions having been described. Most of the inversions are short, and recombination between them is very frequent [Battaglia & Birch (4)]. Some are very widespread and may be found from Florida to Argentina, while others are more restricted. The inversions do not present seasonal fluctuations in their frequencies. Experiments in population cages fed with different yeast have not disclosed any special correlation between chromosomal types and the food used [da Cunha (22)]. Proofs of the adaptive value of the inversions in *D. willistoni* were obtained by da Cunha (20, 22, 23) and Pavan, Dobzhansky & da Cunha (97). Some inversions have been found with frequencies significantly above 50 per cent in natural as well as in laboratory populations. It is interesting to observe that some of the inversions which occur with frequencies above 50 per cent are located in the X chromosome. The X chromosome, although hemizygous in the males, is not therefore homogeneous, as was previously supposed. Da Cunha (20) explained the high frequencies of such X chromosome inversions in terms of heterotic genes with "sex-limited" effects. Since then, Bennett (7) has shown mathematically that polymorphism for sex-linked genes can be maintained by selective differences, even without a higher adaptive value of the heterozygotes.

Further evidence of the sensitivity of the chromosomal types of *D. willistoni* to environmental factors was obtained by da Cunha (22). A good sample of this species was obtained in nature and the descendants were bred for more than a year in a large number of quart bottles. The composition of the population was very much changed in laboratory conditions and some gene arrangements were eliminated. After more than a year in quart bottles, the flies were transferred to population cages and again, in the new environment, changes were produced in the composition of the populations. It is interesting to observe that the changes were parallel in six population cages

fed with different yeasts. All analysis so far carried out has shown that in *D. willistoni*, as in the other species, the chromosomal inversions are adaptive.

Remarkable results are being obtained in the analysis of the frequencies of the inversions in the natural populations of *D. willistoni*. So far the compositions of the populations in 78 localities all over the distribution area have been studied [da Cunha, Burla & Dobzhansky (25); Townsend (127, 128); da Cunha & Dobzhansky (26); Dobzhansky (38); da Cunha *et al.* (27)]. The localities for analysis are chosen on the basis of their ecological characteristics. The degrees of chromosomal variability of the populations are measured by the mean number of heterozygous inversions per individual. The populations vary from a mean of 0.2 to a mean of 9.3 heterozygous inversions per female.

The chromosomal variability of the populations is highly correlated with environmental conditions. The populations in heterogeneous environments are more variable than those living in more homogeneous habitats. The populations living in Central Brazil where the environment is very heterogeneous, rain forest and cerrado being intermingled, have the maximum variability of 9.3 heterozygous inversions per female, while those living in the semiarid caatinga are much more uniform, with 0.8 heterozygous inversion per female. When chromosomal variability is studied in similar environments the differences between the populations are found to be correlated with the abundance of competitors: variability is higher where the competitors are less numerous. The abundance of competitors has been measured on the basis of the frequencies of the sibling species of *D. willistoni*, namely *Drosophila paulistorum* Dobzhansky & Pavan, *Drosophila equinoxialis* Dobzhansky, *Drosophila tropicalis* Burla & da Cunha, and *Drosophila insularis*. The chromosomal variability was also shown to be lower near the margins of the geographic distribution of the species, as well as in the regions near the limits of ecological tolerance. The populations studied in the West Indies showed a correlation between the size of the islands, their distances from mainland and the degree of chromosomal variability. The populations in islands more distant from the continent are less variable than those in the islands closer to mainland. The smaller the islands and the less abundant the ecological opportunities, the lower the degrees of chromosomal variability.

Da Cunha & Dobzhansky (26) and da Cunha, Dobzhansky, Pavlovsky & Spassky (27), using a method devised by the ecologist Dansereau (28), estimated in quasi-quantitative way the degree of ecological diversity of the environments where the samples of *D. willistoni* were obtained. The degrees of chromosomal variability of all the populations, with the exception of three in a same region of Brazil, were shown to be strongly correlated with the degree of heterogeneity of the environments. In other words, the degree of variability is determined by the degree of disruptive selection in the populations [see Mather (93)]. Chromosomal polymorphism is a method by which the populations become more efficient in the exploitation of the en-

vironmental opportunities. Genetical diversification permits the populations to use the diversity of ecological niches available in the environment. The greater the ecological diversity, the greater the chromosomal variability. This explanation of the relation between adaptation of the population and chromosomal polymorphism has been shown to be consistent with the mathematical studies of Levene (71) and Li (90).

The results obtained in *D. willistoni* have been confirmed by the analysis of the chromosomal polymorphism of other species made by Dobzhansky, Burla & da Cunha (39a), da Cunha, Brncic & Salzano (24), Salzano (101) and Carson (14, 15). When closely related species are analyzed, the ecologically more versatile and widespread turn out to be the more variable chromosomally.

We have, in this analysis, to make the distinction between free variability and the variability caused by organized gene complexes. Carson (14) developed, for this purpose, a very useful index of crossing-over for *Drosophila* populations. It gives a very good measure of the evolutionary flexibility of the populations. Carson's own words describe the crossing-over index as:

an expression of the per cent of the total haploid euchromatic chromosome length of the chromosomes of an individual in which free crossing-over may occur. The index is prepared by measuring the euchromatic length of the entire polytene chromosome complement and equating this to 100. The length of the chromosome segments in which crossing-over is blocked is then measured in per cent of the total. The total blocked length is subtracted from 100, leaving the per cent of the total in which crossing-over occurs. A chromosome segment is arbitrarily considered to be "blocked" if crossing-over occurs there in the formation of no more than 1% of the gametes. . . . In the preparation of the index, all the chromosomal section located within a segment heterozygous for an inversion is considered to be blocked for crossing-over. Although it is known that inversions suppress crossing-over in sections of the chromosome adjacent to them, this suppression has not been taken in account, as there is little basis as yet for accurately estimating it.

Analysis of *D. robusta* populations with the crossing-over index gave a result very similar to that found in the studies of the populations of *D. willistoni*. Free recombination occurs least in the central populations, and increases toward the margins of distribution, where it reaches the maximum. As Carson (14) expertly pointed out, the high degree of chromosomal polymorphism implies a decrease in recombination. Populations with high degree of chromosomal polymorphism are highly adapted to the environment but are less plastic than chromosomally less polymorphic populations.

An adaptation to the decrease of recombination produced by the heterozygosis for chromosomal inversions is generally found in *Drosophila*, as in grasshoppers. The heterozygosis for chromosomal inversions produces an increase in the frequency of recombination in the structurally homozygous parts of the genome. Such increases in recombination were analyzed in detail by White & Morley (140) in grasshoppers; by Redfield (99, 100),

Zimmering (144), Schultz & Redfield (102, 103), Steinberg (118, 119), and Steinberg & Fraser (120) in *D. melanogaster*; by MacKnight (91) and Levine & Dickinson (75) in *D. pseudoobscura*; and by Komai & Takaku (68, 69) in *Drosophila virilis* Sturtevant.

In environments close to the margin of the distribution area or close to the limit of the ecological tolerance, such as deserts, "the species cannot afford the luxury of chromosome polymorphism" [Stone (121)]. The advantage of free recombination in coping with the adversity and harshness of environment is at a high premium in such conditions:

Regional differentiation of chromosomal characteristics, with relative structural homozygosity at the margin, thus provides a solution to the ancient evolutionary dilemma with which the organism is continually faced. This is the maintenance of a high adaptive level and at the same time the preservation of a balance between too much recombination, which may destroy adaptive gene complexes, and too little, which restricts the ability of the organism to change genetically in response to drastically changed conditions [Carson (13)].

A species, regarded as a whole, is in the central and optimal environments highly adapted to the environment by means of organized gene complexes, while in the margins of distribution it is less specialized but much more plastic. The marginal populations constitute a source and reservoir of genic combination.

It must be stated that adaptation by means of gene complexes is not obligatory. A species can be successful and present no chromosomal variability. The adaptation by means of gene complexes represents a level of genotypic integration that may be absent in less progressive species. It is interesting to note that in the chromosomally variable populations, the adaptation is based mainly on the heterozygotes. When samples of such populations are removed to very uniform environments, the variability is maintained. Da Cunha (22) obtained a sample from an extremely variable population of *D. willistoni* heterozygous for 28 different inversions, and kept them in the laboratory for more than 30 generations in mass cultures. After that time, the population was still heterozygous for 18 different inversions, despite the uniformity of the laboratory conditions.

The correlation between chromosomal variability and the environment is not so clear in *D. subobscura*, in some domestic species of *Drosophila* and in the populations of grasshoppers studied by White. In *D. subobscura* Stumm-Zollinger (123), Goldschmidt (60), and Kunze-Mühl, Müller & Sperlich (70) did not find any clear correlation of the chromosomal variability of the population with the ecological condition where it lives. The same is true of the Mesophragmatica group of Andean species of *Drosophila* [Brcnc (9, 10)].

Among the domestic species, *D. funebris* presents a variability somewhat similar to that found in the wild species. Dubinin & Tiniakov (46, 47) found in Russia that in any given city the concentration of inversions is higher in

the center and decreases gradually toward the periphery. When comparisons are made between populations of *D. funebris* living in different cities, it is found that the populations living in the larger cities are more variable than the ones living in the smaller cities. Garden cities support less variable populations than industrial cities. The variability of *D. funebris* is correlated with the degree of urbanization. The higher degree of urbanization probably determines increase in the number of ecological niches available for *D. funebris* and, consequently, the higher chromosomal variation.

Cosmopolitan domestic species are very difficult to analyze [Freire-Maia (56, 57); Freire-Maia *et al.* (59)]. Some like *D. ananassae* or *Drophila melanogaster* Meigen, are variable chromosomally, while others, like *Drophila simulans* Sturtevant are not. Since they are transported by man, many different strains are repeatedly introduced in the localities where they live. That makes it completely impossible to analyze relations between variability and environment. In those species historical factors make obscure the genetic adaptation. Carson (14) suggested that the very aggressive and cytologically uniform cosmopolitic species "have evolved from endemic ancestors in marginal situations, and have retained the open systems of recombination characteristic of their progenitors."

White (134, 135, 136), White & Morley (140), White & Nickerson (141) found chromosomal polymorphism in several species of North American grasshoppers. The commonest variants are pericentric rearrangements which suppress chiasma formation inside the inverted segments and increase recombination in the regions distal to the rearrangements [White & Morley (140)]. Such pericentric rearrangements were found in several species of the genus *Circotettix* and *Trimerotropis* and in *Pediocirtetes nevadensis* Thomas. The amount of heterozygosity in *Trimerotropis sparsa* Thomas varies from 0.4 structurally heterozygous bivalent per individual, in Piceance Creek, Colorado, to 3.0 in Lovelock, Nevada [White (135, 136)]. In *Trimerotropis suffusa* Scudder the mean number of heterozygous bivalents per individual ranges from 4.0 in Tahoe, California to 0.28 in Colorado [White (137)]. The most variable species is *Trimerotropis thalassica* Bruner in which 10 pair of chromosomes are variable. Another type of variability found was heterozygosity for chromosome fusions which occur in *Trimerotropis gracilis sordida* Walker, *Trimerotropis cyaneipennis* Bruner, *T. sparsa* and in *Circotettix undulatus* (Thomas).

No correlation was found in the grasshoppers between the chromosomal variability and the environment. No decrease of chromosomal variability was observed toward the margins of distribution, and likewise no correlation between heterogeneity of environment and degree of polymorphism. Very widely distributed species like *Trimerotropis pallidipennis* (Burmeister) are chromosomally uniform, while geographically restricted species like *T. thalassica* are very variable. One of the rarest North American grasshoppers, *P. nevadensis* shows a high degree of chromosomal polymorphism [White & Nickerson (141)]. In the Australian grasshopper *M. scurra*, White (138) was

able to show that the chromosomal polymorphism is heterotic, but, again, no correlation of the variability with the environment was found.

Wahrman (129) and Wahrman & O'Brien (130) reported the presence of chromosomal polymorphism in the populations of mantids. They found that populations of *Ameles heldreichi* Brunner present individuals with 27, 28 or 29 chromosomes. Another species, *Ameles cypria* Uvarov, has two chromosomal genotypes with 25 or 26 chromosomes. Still another species, *Ameles* sp., shows 10 different structural types, seven being present in a small population. The correlations of the cytology of those species with ecological factors were not yet studied.

Ford (55) called attention to a situation which may arise in ecologically or geographically marginal populations. Many times peripheral populations are able to survive in only very limited environments. Ford quotes examples such as those of the moth *Malacosoma castrensis* Linnaeus and of the butterflies *Melitaea cinxia* Linnaeus and *Thymelicus acteon* (Rottemburg). Those species are widespread in continental Europe, but in their margins of distribution, in England, they are restricted to very limited and ecological special areas. In such ecologically and geographically restricted populations, chromosomal polymorphism can be imposed on the populations as an escape from forced inbreeding [Darlington (29, 30)]. This is the explanation given by John & Lewis (66, 67, 87) to the polymorphism for translocations observed in some coal-mine populations of *P. americana*. Chromosomal polymorphism in rare, ecologically or geographically, restricted species may represent only a device to prevent inbreeding and forced homozygosis.

Smith (105, 107, 108) found an interesting instance of chromosomal polymorphism in a coccinellid beetle *Chilocorus stigma* (Say). The populations of this species can be polymorphic for chromosomal fusions. Four fusions have been found in large numbers of individuals in the populations. One individual homozygous for six fusions was recorded. What makes this case especially interesting is the geographic pattern of the polymorphism. Smith analyzed more than 200 males. All those from Florida to Canada proved to be heterozygous for a fusion A. This fusion, present everywhere, is an X_1X_2Y sex-determining mechanism formed by the fusion of the Y of a neo-XY with an autosome. Fusion C was carried by 18 per cent of the males from the Maritimes. The males from Ontario were heterozygous for B or C or for both in 85 per cent of the individuals. The males from Saskatchewan carry A, B, and C fusions and 60 per cent of them had another fusion D. There is, evidently, an increase of polymorphism from the south to north and west. Smith supposes that this polymorphism had its origin after the last glacial period and that it helped the species to colonize the area left by the retreating ice. This hypothesis is supported by the cytological analysis of the closely related species of the genus *Chilocorus* in the west. These species are homozygous for two, three, or six fusions and form a gradient in their geographical distribution. The species with two fusions is the southernmost and the one with six is the northernmost species. We have therefore in the populations of

C. stigma a picture of what happened in the western species before their speciation. Smith (106) and Manna & Smith (92) found polymorphism for chromosomal fusions also in bark beetles of the genus *Pissodes*. Three species studied were found to be polymorphic: *Pissodes approximatus* Hopkins, *Pissodes canadensis* Hopkins, and *Pissodes terminalis* Hopping. Two fusions, giving 9 karyotypes, are present in the populations of *P. approximatus* in Ontario. The analysis made by Manna and Smith suggests that the homozygotes for the fusions are the best adapted types. In *P. canadensis* the reverse seems to be true. The populations of *P. terminalis* showed polymorphism for three fusions.

Studies like these made by Smith show how the comparative cytogenetic analysis of species and of polymorphic populations can contribute to our knowledge of the evolutionary processes.

CONCLUSION

Comparison between closely related species of *Drosophila* which are chromosomally either very polymorphic, somewhat polymorphic, or not polymorphic at all discloses interesting relations between polymorphism and the phenomena used by Thoday to characterize biological progress. The heterozygotes in the polymorphic populations are the most versatile and homeostatic individuals. The more polymorphic species are those which have larger geographical distribution areas and also varying kinds of environments within their distribution area. The species which are chromosomally uniform have a very large genetical versatility but very little genetical stability. The chromosomally polymorphic species have several levels of balance between genetical stability and genetical versatility. They have chromosomally uniform populations which present high degrees of genetical versatility and are a real source of new genetical experiments in adaptation. They have populations very polymorphic chromosomally which are well integrated and stable in their gene pools. They have also all the types of intermediate populations between those very polymorphic and more stable and those chromosomally uniform and very versatile.

The chromosomally more polymorphic species possess more integrated genotypes and maintain the capacity to create new adaptive types. They are simultaneously better adapted to the present environment and more likely to survive temporal changes. They are, therefore, the most progressive species.

Those conclusions are based mainly on the work done in *Drosophila* species which are well known not only genetically but biologically in general. Most work on other organisms supports these conclusions. The seemingly exceptional examples are organisms of which the biology and ecology are little known or are those in which polymorphism is imposed as a means to prevent inbreeding.

The study of chromosomal polymorphism has greatly contributed to the

understanding of the processes of microevolution and will contribute even more when cytological studies are made on larger groups of animals.

As has been shown, populations polymorphic for chromosomal rearrangements constitute an ideal object of study for the understanding of the processes which produce adaptation and progress in evolution. Individual plasticity, genetical stability, genetical versatility, mastery of ecological niches, and diversity of types are all correlated with chromosomal variability. The understanding of adaptation and of evolutionary progress, as defined by Thoday, can be increased more by the study of chromosomal polymorphism than of any other phenomenon.

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THE PHYLOGENY OF COLEOPTERA¹

By R. A. CROWSON

University of Glasgow, Glasgow, Scotland

ORIGIN OF THE COLEOPTERA

There have been two main theories on the ancestry of Coleoptera, the blattoid and the neuropteran. The former theory, as expressed by Handlirsch (30) and Zeuner (71), argues that the elytra and general body form are better indicators of affinity than the mode of development and accordingly seeks to derive beetles from blattoid or protoblattoid ancestors. The alternative theory, supported by Crowson (13) and many others, attaches greater importance to the larval type and the complete metamorphosis, on which criteria the relatives of Coleoptera are to be sought among the Megaloptera and Neuroptera. The fossil evidence adduced for the blattoid theory is chiefly Tillyard's (65) celebrated *Protocoleus* from the Upper Permian of Australia. Forbes (24) concluded from the picture and description alone that *Protocoleus* was essentially orthopteroid; I have been able to study the type in the Australian Museum, Sydney, and can add that Tillyard's picture contrives to suggest a beetlelike convexity in what is a perfectly flat tegmen. The oldest undoubted Coleoptera are recorded from Upper Permian deposits in North Russia (46) and from deposits rather less certainly attributed to the same age in Australia. The remarkable Lower Permian fossil *Tshekardocoleus* (39) looks like an intermediate form between cupedid-type Coleoptera and Megaloptera of the *Corydalid* type and may be taken as direct evidence in support of the neuropteran theory. Other elytra-bearing forms from the Permian, e.g., Blattelytridae and Protelytridae (71), show no convincingly coleopterous features and might equally well be ancestral to modern Dermaptera. The fossil evidence seems to be at least consistent with the theory that Coleoptera took their origin from megaloptera-like ancestors during the Permian period, very soon after the first adaptive radiation of the Endopterygota (33).

SUBORDERS OF THE COLEOPTERA

The time-honoured division into Adephaga and Polyphaga is evidently the expression of a true, deep-rooted phylogenetic cleavage, and, if we exclude from them a very few rare and little-known forms, both divisions appear to be truly natural. The few anomalous forms have more recently been referred to additional suborders, the Archostemata (Cupedidae + Micro-malthidae) of Forbes (23) and the Myxophaga (Lepiceridae + Sphaeriidae + Hydroscaphidae) of Crowson (13). There is direct fossil evidence of the antiquity of Archostemata, fossils resembling Cupedidae being known from the Upper Permian (46), from Triassic, Jurassic, and Cretaceous deposits, and from the Eocene and Oligocene in Europe. Triassic fossil wood with bor-

¹ The survey of the literature pertaining to this review was concluded in May, 1959.

ings from America (69) and Germany (44) is most probably attributable to cupedid-like beetles; no other known type of wood-boring insect has been discovered for periods prior to the Jurassic. The oldest clearly adephagan fossils I have seen are from the Lower Jurassic (Lias), but the group is probably almost or quite as old as Archostemata. The recognition of Polyphaga among early fossils depends on the finding of prothoraces well enough preserved to show the ventral sutures. At present it seems likely that the initial radiation of Coleoptera gave rise to three stocks: one carnivorous (Adephaga); one wood-boring (Archostemata); and a third which may at first have retained the original habitat (under bark) and food (moulds) but soon underwent various reductive changes. First the fusion of tarsus and claw in the larval leg formed a tarsungulus, then perhaps a loss of the special nutritive cells of the ovarioles produced panoistic ovarioles (somewhere about this stage the ancestral Myxophaga presumably split off), and finally the loss of the notopleural sutures of the prothorax and the oblongum of the wing produced typical Polyphaga with their acrotrophic ovarioles (cf. 23). The attempt by Paulian (51) to split Polyphaga into two suborders, Heterogastra and Haplogastra, can hardly be justified either practically or theoretically, since the structure of the second abdominal segment on which it is based is a notoriously inconstant character within many groups.

POSITION OF STYLOPOIDEA (STREPSIPTERA AUCTT.)

The stylopoids (6) are highly adapted for an endoparasitic mode of life; in seeking evidence for their natural relationships it is necessary to discount these adaptations and try to find out what an assumed nonparasitic ancestor of the group may have been like. It appears that no features of the Stylopoidea are inconsistent with a derivation of the group from normal coleopterous ancestors, and several are difficult to reconcile with any ancestry other than this. Most notable, of course, is the use only of the hind wings in flight, but we may recall also the more extensive sclerotization of the abdominal sternites than of the tergites (contrary to the usual endopterygote condition), the nature of the metendosternite (11), and the structure of the first-instar larva, which does not seem to differ from similar "triungulins" of Meloidae and Rhipiphoridae in any character which could possibly be regarded as of ordinal importance. Moreover, the one other coleopterous group in which habits analogous to those of stylopoids are developed, the Rhipiphoridae, shows remarkably similar adaptations in its endoparasitic forms [Rhipidiini (see 60, 71)]. If we accept the Stylopoids as true Coleoptera, the next question that arises is: can they be placed in one of the existing suborders, or will a new one be needed? The first-instar larvae of stylopoids have the polyphagan type of leg, and the absence of a notopleural suture in the prothorax of the adult males suggests a similar relationship, though it may be recalled that *Micromalthus* also lacks these sutures. A very striking feature of adult male stylopoids (and free-living females of the more primitive types) is the absence of functional spiracles from the freely exerted ab-

dominal segment 8, which, as we shall see, is characteristic of a large section of Polyphaga; further discussion of Stylopoidea is deferred till we come to deal with that group.

PHYLOGENY IN ADEPHAGA

Adephagan beetles fall into two traditional sections, Geadephaga and Hydradephaga, the latter group being distinguished essentially by aquatic adaptations. No serious coleopterist has ever suggested that terrestrial caraboids are derived from aquatic ones; it is universally assumed that the derivation has been in the reverse sense. But what were the geadephagan ancestors of Hydradephaga like, and when did they live? Direct fossil evidence offers a little help, in that a fossil [*Actea* Germar (see 28)] looking very like a large dytiscid is described from the Upper Jurassic of Germany; we can feel fairly certain that the origin of Hydradephaga was at least older than this: and that they probably originated near the beginning of the caraboid stock and possibly go back to the Triassic. One feature of the Hydradephaga that is not a very obvious adaptation to aquatic life is the lateral extension of the hind coxae to meet the elytral epipleura, thus separating the metapleura from the first visible abdominal sternite. This feature occurs in only one group of the Geadephaga, the Trachypachini [Trachypachidae (13)]. The same group shows several other hydradephagan-like features, e.g., the antennae with all the segments more or less glabrous, and the metepisterna impinging on middle coxal cavities as in Dytiscidae-Complicati (62). Also, it belongs in that section of the Geadephaga, the Isochaeta of Jeannel (38), in which both spurs of the front tibiae are truly terminal, as they are in all Hydradephaga. The Trachypachini are evidently a relict group, and it seems quite reasonable to regard them as remnants of a Geadephagan group ancestral to Hydradephaga. Unfortunately, nothing is known of their larvae and little of their habits, other than that they are liable to occur in rather wet forested places. In other groups of Coleoptera (cf. Hydrophiloidea and Dryopoidea), aquatic adaptations do not seem to have taken place concurrently in adults and larvae, and on this analogy we might expect at some stage in the ancestry of Hydradephaga to find forms with aquatic adults and terrestrial larvae or vice versa.

In place of the Geadephaga-Hydradephaga division, various authors (3, 13, 29, etc.) have proposed to separate one or more of the families Gyrinidae, Paussidae, and Rhysodidae into superfamilies distinct from Caraboidea. The special features of gyrinids are apparently caenogenetic and connected with their peculiar mode of life; if these are discounted, there is really very little to separate Gyrinidae from Noteridae or Dytiscidae (31), and in phylogenetic classification I can see no grounds for giving the Gyrinidae a higher status than these two families. The position of Paussidae is rather different, in that at least one larval feature of *Paussus*—the presence of a movable lacinia mobilis on the mandible—is one that might be taken as primitive (3, 19) and does not occur in other caraboids. However, larvae of

the ozaenine genus *Physea* [van Emden (18)] are very like *Paussus* larvae but have a normal rigid retinaculum on the mandible like other carabids, suggesting that the lacinia mobilis of *Paussus* may be secondary. As pointed out by van Emden (18) and Darlington (14), the paussids are very closely linked with Ozaeninae, hitherto included in Carabidae; if Paussidae are redefined to include the ozaenines (13, 38) a more natural grouping results. The Rhysodidae, like the gyrinids, are highly modified for a very unusual (at least in Adephaga) mode of life; if we discount these modifications, they seem to be related to normal Carabidae in much the same way as Gyrinidae are to normal Dytiscidae. Rhysodids have, in fact, almost all the special characters of Jeannel's *Simplicia* (38). The habitus of rhysodids is very characteristic and should make them readily recognizable as fossils; unfortunately, no fossil rhysodids have yet been described. From the gut contents and mode of occurrence of at least the New Zealand species, I can assert with some confidence that they are not, as I had formerly assumed, carnivorous (13); the real food appears to be bacteria-rich, decaying wood.

The major recent contribution to the phylogenetic classification of Carabidae has been made by Jeannel (38), who has not, however, given any opinion on the functional significance of the major changes postulated in the evolution of the group; the same author has attempted to assign times and places to the origin of various carabid groups on the basis of highly speculative theories about palaeogeography. The recent work of Sharova (61) has drawn attention to various adaptive features of carabid larvae; work on these lines may be expected to throw important light on carabid evolution.

In the Hydradephaga, a distinction is fairly readily drawn between the three families of "lower" types (Amphizoidae, Hygrobiidae, and Haliplidae) and the three "higher" ones (Noteridae, Dytiscidae, and Gyrinidae). The higher families presumably shared a common ancestor which might have been an extinct form related to *Amphizoa* and *Hygrobia*. We may imagine two adaptive radiations of Hydradephaga: an early one may have given rise to Haliplidae (herbivorous), Hygrobiidae (bottom-feeding carnivorous), Amphizoidae (surface-feeding carnivorous), and ancestral Dytiscidae (free-swimming carnivorous); from the ancestral dytiscid stock a second radiation may have produced Noteridae (herbivorous), Gyrinidae (carnivorous, adult surface-feeding, larva bottom-feeding), and Dytiscidae proper.

THE MAJOR DIVISION OF POLYPHAGA

The special features distinguishing Polyphaga from Archostemata, etc., are essentially structural simplifications: the fusion of tarsus and claw in the larval leg, the loss of a cross vein in the wings, a simplified suture in the prothorax, and probably the loss of the original nutritive cells in the ovarian follicles, giving rise to panoistic ovarioles and thence to acrotrophic ones. Simplifications of this type are apt to accompany a considerable reduction of body size, suggesting that the original Polyphaga may have been very small insects, as are the existing Myxophaga and such primitive types as

Clambidae. If so, it is rather strange that the basic Polyphagan wing venation shows so little reduction compared with that of Cupedidae; it may be necessary to postulate that a reduction of wing venation as a result of great reduction of body size is not altogether irreversible.

The most notable recent advance in our understanding of the relationship of polyphagan beetles has been the gradual realization that a large section of the suborder may be set apart from the rest by the following combination of characters: (a) adult—Malpighian tubules cryptonephridic, eighth abdominal segment without functional spiracles, hind coxae never excavate, aedeagus with tegmen of cucujoid type or derivable from it, metendosternite of hylecoetoid type or derivable from it; (b) larva—maxillary mala never split into two pointed lobes nor with articulated galea, spiracles with normal closing apparatus, ninth abdominal segment never with articulated urogomphi. This division, which has been named Cucujiformia (13), includes more than half the species of Coleoptera; it includes the Rhynchophora and Phytophaga of earlier authors, together with Heteromera, most of the Clavicornia, some of the Malacodermata, and perhaps also the Strepsiptera. Apart from the last-mentioned group, in which the adult and larval structure are so modified as to make ambiguous several of the leading characters of Cucujiformia, the series seems to be perfectly clearly defined; all Coleoptera which have been adequately studied are either definitely Cucujiformia or definitely not. Although foreshadowed to some extent in the work of Sharp & Muir (63) and Forbes (22, 23), the series first took clear shape in the work of Poll (53) on the Malpighian tubules. Böving & Craighead's (3) larval system had united Clavicornia with Heteromera, but did not associate them with the Phytophaga-Rhynchophora; evidence in support of such an association was forthcoming in the work of Mansour (45) on the formation of the adult mid-gut. Peyerimhoff (52) had revised the Böving-Craighead system to bring it more into line with the results of Poll and others. Further evidence of similar import came from the study of Crowson (10) on the metendosternite, and in the same year the veteran coleopterist Lameere revised his earlier system to incorporate a division ("Cryptonephridiés") practically equivalent to Cucujiformia. In proposing the latter name, Crowson (13) first drew attention to the lack of spiracles on abdominal segment 8 in the adults.

Apart from Cucujiformia, several other major series may be distinguished in Polyphaga, the best marked of them being, perhaps, Staphyliniformia and Elateriformia. The Staphyliniformia are usually separable from other Polyphaga by the presence of articulated appendages (urogomphi) at the back of the ninth abdominal tergite in the larva (the few members of the series in which these are lacking are evidently related to forms with them). The larval spiracles have a normal closing apparatus and the adults have four or six Malpighian tubules with free ends. The articulated urogomphi have often been homologized with cerci (19), and most authorities (e.g., 3) have regarded them as an ancestral feature of Polyphaga and of Coleoptera gen-

erally; if either of these views is correct, the urogomphi cannot be regarded as a satisfactory defining character for Staphyliniformia. Against the homology with cerci, it may be urged that the urogomphi belong clearly to segment 9, and at least in higher Staphylinidae (Staphylininae) apparent correspondence may be traced between larval and pupal urogomphi and similar articulated structures in the adults which are evidently the homologues of paraprocts (pleurites of abdominal segment 9) in other beetles; cerci on the other hand are true segmental appendages which on segment 9 are represented by the gonapophyses. Against the primitive nature of articulated urogomphi in beetle larvae we may note their absence at any stage in the known archostematan larva (*Cupes* and *Micromalthus*) and their absence in the only known myxophagan larva (*Hydroscapha*), in most Carabidae, in such primitive Polyphaga as the Eucinetodea (*vide infra*), and in related orders such as Megaloptera (where any analogous organs are situated on segment 10 and more ventrally). Furthermore, articulated and muscled dorsal appendages occur on abdominal segments 1 to 8 in the larvae of *Sialis*, gyrinids, etc., where they are normally regarded as secondary; if such things can be secondarily evolved on other abdominal segments, why not on segment 9?

In the Elateriformia, the larval spiracles usually lack a closing apparatus, the larval mandibles lack a molar part and the maxillae have a fingerlike articulated galea; the adult normally has excavate hind coxae, filiform to serrate or pectinate antennae, functional spiracles on abdominal segment 8, a more or less trilobe aedeagus (63), and four or six free Malpighian tubules. Standing outside the series so far defined we have the old Lamellicornia (Scarabaeoidea), the Terebrantia (Bostrychoidea), and a number of odd families, the Dascillidae, Helodidae, Eucinetidae, Clambidae, Derodontidae, Nosodendridae, Dermestidae, Thorictidae, and Jacobsoniidae (*Sarothrias*). The Dascillidae *s. str.* (3, 13) appear to be related to Scarabaeoidea, and a series Scarabaeiformia (*q. v.*) may be defined to include Dascilloidea (containing only Dascillidae *s. str.*) and Scarabaeoidea. The next three families listed appear to be more closely related to each other than to anything else, and I hereby propose for them the new superfamily Eucinetodea, defined essentially as follows: (a) larva—a mandibular mola, maxillae with distinct but not articulated galea and lacinia, spiracles with normal closing apparatus, urogomphi absent, and abdominal tergites and sternites similarly sclerotized; (b) adult—aedeagus with the parameres not distinctly articulated, male ninth abdominal segment with the pleurites fused together in front of the tergite, metendosternite characteristic (11). The last five of the listed families were united in the superfamily Dermestoidea (13), which with Bostrychoidea formed the series Bostrychiformia [Crowson (13)]. Both Eucinetodea and Dermestoidea appear to include particularly primitive types of Polyphaga. It seems possible that the first adaptive radiation of the polyphagan stock, probably in the Triassic period, gave rise to three ancestral stocks: staphyliniform, eucinetoid, and dermestoid. From the eucinetoid stock we may imagine a dascillid-like ancestor developing which by the

beginning of the Jurassic might give rise to ancestral Scarabaeiformia and Elateriformia. The dermestoid ancestor presumably gave rise to an ancestral cucujiform stock in the early Jurassic period and perhaps later to the Bostrychoidea. The most direct way in which this hypothesis might be checked is by the discovery of really well-preserved Triassic or Jurassic fossil Coleoptera.

SERIES STAPHYLINIFORMIA

The phylogenetic relations between the three superfamilies [Histeroidea, Hydrophiloidea, Staphylinoidea; (13)] included here are by no means evident or generally agreed. Judging from the wing venation, it would appear that Hydrophiloidea include the more primitive forms of the group, but there may be some doubt about the strict applicability of Dollo's Law to wing venation in Polyphaga. The hydrophiloids would appear to be primitive in several other respects, e.g., the six free Malpighian tubules, the simple trilobe aedeagus, the nature of the larval maxillary lobes (if Hydraenidae are accepted as hydrophiloid), and perhaps the cephalic egg bursters of the first-instar larvae. But the aquatic habits of the adults, and correlated peculiarities of the antennae (35) are unlikely to be primitive for Staphyliniformia as a whole. Furthermore, there is little beyond these aquatic modifications of the adults to link hydraenids with Hydrophilidae proper, and if Hydraenidae are excluded, the basic characterization of the superfamily will be considerably altered. A number of authors, including Böving & Craighead (3), Forbes (23), Paulian (51), and Peyerimhoff (52), have transferred hydraenids to Staphylinoidea, attributing their resemblances to hydrophilids to convergent development in the adults. Support for this mode of thought could be adduced from the structure of the aedeagus, the wing venation and folding, and probably the Malpighian tubules of the adults, also from the general head structure of the larvae; against it, in addition to the special aquatic adaptations of the adults, there are the egg cocoons (4) and cephalic egg bursters (20) of hydraenids, and perhaps the fact that in at least some hydraenid larvae the galea shows some indication of coming from the palpiger. The association of Histeridae with hydrophiloids, proposed by Böving & Craighead (3) and supported by van Emden (19), rests on the remarkable larval similarity between *Helophorus* and histerids; it is not supported by any important feature of the adults and is opposed by at least one probably important larval feature—the presence of abdominal egg bursters in at least one histerid larva (20).

If the hydraenids are hydrophiloid, as supposed by the leading recent specialist of the group, d'Orchymont (49), we must assume that in wing venation and folding, as perhaps in the aedeagus, hydraenids show the effects of a great reduction in size and probably a change in body form. A hypothetical ancestor of hydraenids and hydrophilids would probably be about the same size (and possibly shape) as the modern *Spercheus*. It would have been herbivorous in both larval and adult stages, feeding presumably

on some sort of alga; it may have had the beginnings of a ventral plastron, antennae with five glabrous segments before the cupule and five densely tomentose segments after it (35, 49), wings spercheid- or hydrophilid-like, and a larva somewhat like that of existing hydraenids. The key factor in the initiation of the spercheid-hydrophilid line would be the switch to carnivorous larval habits, with a concomitant tendency to increase rather than decrease body size, followed by a trend to more perfect aquatic adaptation of the larvae. In the hydraenid line, evolution would seem to have been in the direction of more perfect plastron respiration and a reduction of body size. The general retention of herbivorous habits, and such associated features of the mouth parts as a mandibular mola, in adult hydrophilids is an example of what I believe to be a general tendency in Coleoptera: greater conservatism of the mouth parts in adult beetles, the imagines lagging behind the larvae in adaptations to new types of food.

In the Histeroidea, *Sphaerites* doubtless represents the most primitive living type; its larva, like that of the rather less primitive *Syntelia*, is still undescribed. The basic imaginal features of histeroids—completely retracted eighth abdominal segment without functional spiracles, truncate elytra leaving tergite 7 uncovered, wing venation and folding (22, 23)—offer parallels with the cucujoid Nitidulidae. In the Histeridae, the known larval habits are predacious like those of hydrophilids, and the evidence to date indicates that the adults likewise are mainly carnivorous; the differences between the mouth parts of adult Histerinae and *Sphaerites* parallels that between the carnivorous and the fungivorous Trogositidae.

The Staphylinoidea are second only to Cucujoidea in adaptive diversity and present similarly difficult problems for the phylogenist. First, there is the problem: what is (or was) a primitive staphylinoid like? My own suggested answer to this question is: the genus *Empelus* LeConte. In this genus the hind coxae are excavate with narrow femoral plates extending their whole length, the tarsi are simple and five-segmented, the antennae have a normal three-segmented club, the mandibles are similar to those of other primitive (and fungivorous) Polyphaga, the maxillae have distinct galea and lacinia, the gular sutures are separate, the front coxal cavities open behind, the front coxae have exposed trochantins and project slightly, the metendosternite is similar to that of primitive anisotomids (the aedeagus being of the trilobe type with well-marked basal piece, the wings being normally staphylinoid), the abdomen has six visible sternites (segment 8 fully exerted), and the elytra have distinct epipleura. Almost all these characters seem likely to be primitive in Staphylinoidea; there is, however, at least one probably primitive character in the group not found in *Empelus*—the presence of paired dorsal ocelli. *Empelus* has been placed in Clambidae by most of the few authors dealing with it, including Hatch (32); but it was shown not to have the characters of that family by Crowson (13) and was excluded by Endrödy-Younga (21). From an *Empelus*-like ancestor, the Ptiliidae (and Limulodidae) might be directly derivable via forms like *Nossidium*. The

Anisotomidae (including Catopinae, Coloninae, etc.) could well come from the same ultimate ancestor. Most of the remaining staphylinoid families could be directly or indirectly derived from anisotomid ancestors of some sort: e.g., the Scydmaenidae as a predaceous development (chiefly on mites) from something like Camirinae (Camirinae auctt.); the Leptinidae as a commensal-parasitic line from near Catopinae (51); the Silphidae from something related to *Hydnobius* or perhaps *Catopocerus*, via forms like *Microsilpha*; the Staphylinidae and Scaphidiidae from silphid types near *Pteroloma* or Agyrtini. The Pselaphidae seem likely to have taken their origin from a primitive member of the staphylinid group Steninae (including Euaesthetinae) as a parallel adaptation to Scydmaenidae for mite predation. The position of Micropeplidae and Dasyceridae (13) is somewhat obscure.

Within the Staphylinidae *s. str.*, a progression may be traced from the slow-moving primitive types, tending to saprophagous habits, e.g., Omaliinae, Oxytelinae, to the highly active, free-living, predatory higher types like Staphylininae and Paederinae, but the detailed relationships within the family are complex and obscure at present.

SERIES SCARABAEIFORMIA

As previously suggested, the series Scarabaeiformia may include the Dascilloidea as well as the Scarabaeoidea. The former group is comprised of only the family Dascillidae *s. str.* with the genera *Dascillus* and *Coptocera* and one or two close allies, possibly also *Genecerus* and *Anorus*; of these only *Dascillus* has a known larva. In imaginal characters, the genus *Dascillus* would appear to be much more primitive than any Scarabaeoidea, in, for example, the wing venation, coxal structure, Malpighian tubules, antennae; if the scarabaeoids were derived from an ancestor with characters like those of *Dascillus*, we must assume an evolutionary sequence which involved a major change in imaginal habits and adaptations but a much less marked change in the larval stage. The adult *Dascillus* is short-lived, diurnal, free-living, and floricolous; whatever the habits of the ancestral adult scarabaeoid were, they could hardly have been like this (though adult habits like those of *Dascillus* occur quite commonly in higher modern Scarabaeidae, e.g., *Phyllopertha*). The larva of *Dascillus* feeds primarily on humus-rich soil and perhaps partly on plant roots; the adult, having only a short ovipositor, presumably deposits its eggs in the surface layer of the soil. The *Dascillus* larva differs from most scarabaeoids in having powers of ordinary walking. A reasonable assumption is that the ancestral scarabaeoid had adopted a mode of life in which its larva did not need to use its legs for walking; the adults probably placed the eggs on or close to an adequate food supply. If this food was subterranean, the adult, lacking a long ovipositor, would have needed burrowing powers to reach it, and the basic features of adult scarabaeoids are suggestive of burrowing adaptations: for example, the enlarged front coxae with spinose tibiae, the compact body form, firm and usually glabrous integument, and the retractable antennae. All these features are closely

paralleled in Anisotomidae-Liodini, which as far as known feed on subterranean fungi. These Liodini show some approach to the typical lamellicorn type of antenna, which is designed (if the teleology may be pardoned) to meet the following requirements: (a) a large area of highly sensitive olfactory sensillae giving maximum sensitivity in detecting a specific odour; (b) directional olfaction in calm air (e.g., near the ground), hence concentration of the sensillae on a pair of movable clubs; (c) ability to protect the sensitive areas while burrowing in the soil. An additional point in favour of subterranean fungi as the ancestral food of scarabaeoid larvae is that larval Liodini [(41); personal observation] differ from normal anisotomid larvae in much the same way that scarabaeid larvae differ from *Dascillus* larvae, except that the curvature of the fleshy body is dorsal instead of ventral. On this theory it would seem likely that ancestral scarabaeoids were comparatively small insects, like the existing Liodini. The facts that the basic wing venation in scarabaeoids is considerably reduced in comparison with dascillids and is as well developed in the small forms of the more primitive groups (e.g., *Ochodaeus*) as it is in the largest types may be taken as supporting evidence.

Within the Scarabaeoidea, the relationship of the existing groups are by no means always evident. If, as seems reasonable, we take the 11-segmented adult antenna of geotrupids as primitive, we shall have to assume either (a) that the reduction to 10 segments is polyphyletic, (b) that the scarabaeid type of abdomen (with six visible sternites, hard tergites, and a telescoping respiratory movement) has developed independently in Geotrupidae and Scarabaeidae, or (c) that the normal polyphagan abdomen (with five visible sternites, soft tergites, and no telescoping movement) of lucanids, trogids, etc., is secondary. It is not easy to choose between these alternatives at present. The Passalidae, it has been suggested (13), are a direct offshoot of Lucanidae, specialized for a peculiar mode of life; the Lucanidae themselves would seem to be related to the remaining Scarabaeoids indirectly by way of Trogidae, the latter group linked with Acanthoceridae by means of *Glaresis*, which is surely one of the most primitive of existing Scarabaeoids. The geotrupids would seem to be linked with scarabaeids proper by way of *Taurocerastes* and Hybosoridae (50). The genus *Ochodaeus* seems to be an isolated and primitive type, allied perhaps to Hybosoridae. Data on the larvae and habits of such forms as *Taurocerastes*, *Glaresis*, and *Ochodaeus* would be of the highest interest and importance for our understanding of scarabaeoid phylogeny. Within Scarabaeidae, it would seem that Glaphyrinae represent the laparostict stock from which Pleurosticti arose. The oldest fossil scarabaeoid known seems to be from the Upper Jurassic [*Geotrupoides* Deichmüller (28)]; distinctive scarabaeoids have not been found among the rich Lower Jurassic beetle faunas.

SERIES ELATERIFORMIA

The new name Elateriformia is here proposed for a series equivalent to the Dascilliformia of Crowson (13) minus the Dascilloidea. In the general

and probably primitive lack of a closing apparatus to the larval spiracles, Elateriformia resemble Scarabaeiformia, with which they may share a common dascillid-like ancestor; the most primitive Elateriformia would probably be those forms most resembling Dascillidae, e.g., Byrrhidae, *Eulichas*, and Psephenidae. Note that the latter two groups have aquatic larvae; the loss of the spiracular closing apparatus is, according to Hinton (37), liable to occur in forms with subaquatic or markedly hygrophilous habits. It seems quite possible that the ancestral Elateriformia had more or less aquatic larvae, which may have fed on some kind of alga (though the loss of the mandibular mola and the articulated galea might prompt comparisons with carnivorous forms). It is most usual for Elateriformia, as probably for Scarabaeiformia, to have long-lived larvae and short-lived adults (many of which probably do not take protein food); by contrast, in Adephaga, Staphylinoidea, and Cucujiformia, it is usual for the larval development to be short and the adult life long. In the Elateriformia it seems that the adaptations of the larvae have, as a rule, been more important than those of the adults; note that in dryopids it was evidently the larvae which took the lead in adopting aquatic habits, whereas in hydrophiloids it was evidently the adults. The major divisions of Elateriformia are, as a rule, better defined on larval than on adult characters.

The Byrrhoidea may represent a direct adaptation of an early Elateriform type to feeding on terrestrial mosses, and they stand rather apart from the rest of the series. The Dryopoidea are a heterogeneous assemblage of the more primitive elateriform types and, as at present defined (3, 13), probably would have included the ancestors of all the remaining superfamilies—Buprestoidea, Rhipiceroidea, Elateroidea, and Cantharoidea. Logically, we should either split the Dryopoidea or merge all these superfamilies into an enlarged Elateroidea which would then become comparable in adaptive diversity with Staphylinoidea or Cucujoidea. Within the Dryopoidea, there is some indication of two main lines: one proceeding via Psephenidae to Heteroceridae, Dryopidae, Limnichidae, and Elmidae (36), characterized larvally (except in higher Elmids) by the presence of five or six lateral ocelli; and the other including *Eulichas*, Ptilodactylidae, Chelonariidae, and Euryopogonidae, whose larvae have only a single (often large) lateral ocellus. The essential features of the second group are also found in Rhipiceroidea, Elateroidea, and Cantharoidea. The Buprestoidea are an isolated group with various anomalous characters, e.g., cryptonephridic Malpighian tubules (53, 64), larval spiracles with a functional closing apparatus, larval head with an endocarina, etc. In some of these respects the Buprestoidea resemble the elateroid Eucnemidae whose larvae likewise bore in comparatively dry dead wood. Many features of Buprestids, e.g., the free labrum and well-developed cardines of the larvae, also the three lateral ocelli of *Schizopus* (55) and the characteristic transverse suture of the adult metasternum (11), separate them sharply from Elateroidea and tend to connect them with the dryopid section of Dryopoidea. It is not known whether the larval spiracular

closing apparatus occurs in the supposedly soil-inhabiting larva of *Schizopus* (55). On the analogy to Eucnemidae, I think the closing apparatus has been secondarily redeveloped in buprestid larvae as an adaptation to living in dry dead wood. The Rhipicerioidea seem to be closely linked to the ptilodactylid-chelonariid stock by forms like the New Zealand *Brounia* Sharp. A close affinity between Elateroidea and Cantharoidea, foreshadowed by Böving & Craighead (3) and Forbes (23, 24), became explicit in Lameere (43) and Peyerimhoff (52); there is considerable plausibility in the suggestion (3) that both groups share a common ancestor near the Eurypogonidae. Carnivorous larval habits are universal in Cantharoidea and not uncommon in Elateroidea, and might be postulated in a common ancestor of the two groups; one of their basic common features, the replacement of the larval labrum by a toothed nasale, is found elsewhere in Coleoptera only in basically carnivorous groups (Caraboidea; Hydrophilidae, Histeridae, Staphylininae-Paederinae).

Within Elateroidea, the Eucnemidae show marked larval parallels to the Buprestidae, e.g., in the loss of the legs, reduction of the head capsule, form of the soft, fleshy body, and presence of a spiracular closing apparatus; however, they are connected to Elateridae by various annectant forms, like *Trixagidae*. In these forms, the larvae still have legs and the head capsule is less reduced. The genus *Pactopus* (which has recently been recognized as a fossil in Lower Eocene deposits and thus seems to be a relict type) seems to connect *Trixagus* via forms like *Drapetes* and *Balginae* to normal Elateridae, where the larval spiracles have no closing apparatus. It has been pointed out by Hinton that in *Eulichas* (37) and Elateridae (personal communication), a muscle attaches to the end of the atrium of the larval spiracles at the point where a closing apparatus would be normally; might not this muscle provide the basis for the redevelopment of an effective closing apparatus? It is unfortunate that the larvae of *Anischia*, *Cerophytum*, *Perothops*, and *Phyllocerus* are undescribed, for some of them may represent significant links in elateroid phylogeny.

Among Cantharoidea, the relict type of geographical distribution and elateroid-like adult characters of *Brachypsectra* suggest that this genus is among the most archaic of existing types, while the world-wide abundance and distributional continuity of Cantharidae (*s. str.*) probably indicate the most advanced family of the group. The Lycidae likewise appear to be "modern," with Homolisiidae, Drilidae, Karumiidae, etc., also *Telegeusis* (which certainly belongs in Cantharoidea) representing older and more or less relict types. The peculiar distribution patterns of Drilidae and Homolisiidae probably result from the very low mobility of their wingless females.

The Rhipicerioidea include Callirhipidae [Rhipiceridae (3)] and Rhipiceridae [Sandalidae (3)]; both larval and adult characters of callirhipids emphasize their affinity to the Ptilodactylidae. A callirhipid larva examined by Crowson had no closing apparatus to its spiracles, and in this connection we may recall van Emden's statement that these larvae have been found

only in very moist decaying wood (16). The only known rhipicerid larva, that of *Sandalus* (3), is parasitic on immature Cicadas and exhibits considerable structural degeneration.

SERIES BOSTRYCHIFORMIA

The concept of a special relationship between Dermestidae and the old Teredilia (=Bostrychoidea) was in large part formulated by van Emden (20), though adumbrated by Crowson (10), Forbes (23), Poll (53), and Sharp & Muir (63). The dermestids have more recently been made the type of an independent superfamily (13), corresponding to Meixner's (47) Brachymera minus the Byrrhidae. Various groups that have at one time or another been included in Teredilia, like Lymexylidae, Cisidae, or Sphindidae, are now excluded from Bostrychiformia altogether. Within the series, the Dermestoidea seem to include all the more primitive forms, and it is very probable that Bostrychoidea are descended from dermestoid ancestors near Dermestidae and Thorictidae. The families included in Dermestoidea have been widely separated in many previous systems: Derodontidae have been usually associated with the cucujoid Clavicornia, Nosodendridae associated with Byrrhidae [or Dascilloidea (3)], Thorictidae generally with Clavicornia, Dermestidae with Cleroidea by Böving & Craighead (3), Jacobsoniidae (*Sarothrias*) either with Colydiidae (27) or Rhysodidae (34). The Byrrhidae, included in these forms by Meixner (47), are immediately separable by the lack of a closing apparatus to the larval spiracles, the articulated larval galea, and the adult metendosternite (11).

The most primitive of living Dermestoidea would seem to be the Derodontidae, notable for the possession, at least in *Peltastica*, of what appear to be true adult dorsal ocelli and, at least in *Laricobius*, of cephalic egg bursters in the first-instar larva (25), otherwise known only in Adephaga and Hydrophiloidea among Coleoptera; these two characters occur together only in derodontids and Hydraenidae. The possibility of some direct relation between derodontids and Staphyliniformia can hardly be excluded. In *Derodontus* (personal observation) and *Laricobius* (25), as also in *Nosodendron* (53, 64), there are six Malpighian tubules with free ends, whereas in those dermestids which have been studied (*Orphilus* is not among them) the ends of all six Malpighian tubules are enclosed in a sort of sac and attached to one side of the hind-gut, as they are in bostrychoids. All recent study has emphasized the close relationship of Thorictidae to *Dermestes* [(cf. 2); *Thaumaphrastus* = *Thorictodes*].

The position of Jacobsoniidae (*Sarothriidae* (13)) must still be taken as somewhat speculative. The larva of *Saphophagus* (Crowson, in press) is essentially dermestoid in character, notably in having strongly sclerotized thoracic and abdominal terga combined with soft membranous sterna, but the flat hind coxae of the adult together with its metendosternite and general *habitus*, are more suggestive of Cucujoidea, while the wing venation is completely staphylinoid (the fringe of the larval galea also being suggestive of

Staphylinioidea). *Sarothrias* (*Jacobsonium*) is much more specialized as an adult than *Saphophagus*; its larva is undescribed. It seems perfectly possible that the entire cucujiform complex took its origin from a dermestoid type more or less resembling *Saphophagus*; in any event, I think any general phylogenetic studies in Polyphaga would do well to pay special attention to Dermestoidea.

The special features of Bostrychoidea are doubtless related to a special ancestral mode of life, the larva becoming adapted to bore in dead wood and the adult developing a long ovipositor in order to deposit eggs in crevices, etc., of dead trees. The nature of the relationship between the two main existing families—Bostrychidae and Anobiidae—is obscure at present, but it seems highly probable that Ptinidae are a specialized offshoot from a primitive anobiid type (probably near *Hedobia* and *Eucrada*) and that Lyctidae are a similar offshoot of primitive bostrychids. If resemblance to dermestoids is taken as an indicator of primitiveness in Bostrychoidea, we might suppose the genus *Hendecatomo* to be primitive among existing forms; unfortunately its larva is as yet undescribed. The adult *Hendecatomo* lacks the excavation of the hind coxae which is characteristic of Anobiids as it is of Dermestoidea; the possibility needs to be considered that the excavation in anobiids is a secondary one, the actual form of anobiid hind coxae differing considerably from that in Dermestoidea. Lesne (73, 74) has suggested that *Chilenius* and *Euderia* are primitive bostrychid types; Gardner (72) and others have made similar claims for Dysidinae.

SERIES CUCUJIFORMIA

The enormous Cucujiformia group includes considerably more than half the species of Coleoptera and, hence, may reasonably be regarded as the most highly evolved series of the order. In conformity with this, its basic characters include more derivative features than those of any other comparable group; we may note the cryptonephridic Malpighian tubules, cucujoid type of aedeagus, hylecoetoid type of metendosternite (11, 12), the undivided mala of the larval maxilla, lack of spiracles on abdominal segment 8 of the adult, and so on. The only outside group to which I can see any clear indication of its relationship is the Dermestoidea and particularly the genus *Saphophagus* (*vide supra*). Nevertheless, even in recent times other authors have postulated relationships between one or other cucujiform family and some other series. We may recall those who have maintained the old conception of Malacodermata, with its implied connection of the cleroid families Cleridae and Melyridae with the elateriform Cantharoidea, e.g., van Emden (19) and Meixner (47). There are also those who have tried to establish affinities between certain Cucujoidea and Staphyliniformia, notably Forbes (23), who associated Nitidulidae with *Sphaerites* on wing structure, and Böving & Craighead (3). The last-mentioned authors postulated a derivation of Chrysomeloidea, together with Curculionoidea, Meloidea (= the cucujoid families Meloidae & Rhipiphoridae), Cleroidea, Bostrychoidea, etc., from

byrrhoid ancestors while attaching the main body of Cucujoidea, by way of Derodontidae and *Eucinetus*, to ultimate staphylinoid ancestors. This conception of a fundamental cleavage in Polyphaga was criticized by Peyerimhoff (52).

A particularly interesting question concerning Cucujiformia is whether the Stylopoidea could be included in the series. The established superfamilies here are Cleroidea, Lymexyloidea, Cucujoidea, Chrysomeloidea, and Curculionoidea; there is nothing to suggest a special affinity of stylopoids to the latter two, so we may narrow our consideration to cleroids, lymexyloids, and cucujoids as possible allies. In favour of Cleroidea are the usually predacious habits of their larvae, it being commonly supposed that endoparasitic habits are likely to arise by way of ectoparasitic from predacious habits. In favour of Cucujoidea, there is the marked similarity between stylopoids and some of the more modified endoparasitic rhipiphorids (56, 60). The argument for a connection between stylopoids and Lymexyloidea rests, paradoxically, mainly on the comparison with Cucujoidea. There are two cucujoid families in which parasitic habits together with a "triungulin" type of first-instar larva have arisen, the Meloidae and Rhipiphoridae; the present indications are that parasitic habits have arisen independently in the two groups and that each has its relatives in another family whose larvae usually develop in dead wood. The Rhipiphorids seem to be derivable from Mordellidae, whose larvae live either in dead woody (*Mordella*) or rather herbaceous (*Mordellistena*) stems; this circumstance prompts a comparison with Hymenoptera, with Oryssidae (and Apocrita?) which are most closely allied to Siricidae and Cephidae (70). There may be a proclivity for endoparasitism to develop in certain types of borers. If we compare rhipiphorids with Stylopoidea, the most obvious difference is that parasitic modifications have gone a good deal further in the latter group, with the clear implication that parasitic habits in stylopoids are much older than in rhipiphorids. If the parallel holds, we should look for the relatives of Stylopoidea in a wood-boring type older than the mordellids. The most likely example of such a stock in Cucujiformia is the family Lymexylidae, whose larvae show considerable parallelism with those of Mordellidae (also of Siricidae, etc.). Adult lymexyloids have some characters which might be postulated in an ancestor of stylopoids, e.g., the filiform 5-5-5 tarsi (as in mengeids), the freely projecting front coxae, the somewhat "Hologastran" abdomen, and, even in *Atractocerus*, a great reduction in the elytra, pointing in the direction of the so-called "halteres" of stylopoid males. Of course, if Stylopoidea are really descended from a lymexyloid ancestor, we shall have to postulate a fairly lengthy chain of "missing links," presumably long extinct, to correspond with the lower types of existing rhipiphorids. One or two fossil stylopoids (genus *Mengea*) from the Baltic amber (Lower Oligocene) resemble the most primitive existing types (*Mengenilla*, *Eoxenos*) and permit the deduction that the origin of Stylopoidea is to be sought further back, probably in the Cretaceous era.

There are grounds for supposing that the Chrysomeloidea and Curculio-

noidea are more closely allied to each other than to the remaining Cucujiformia, e.g., the frequent and probably primitive absence of side margins to the prothorax in both, the tarsal structure, the tegmen of the aedeagus lacking articulated parameres, and the basic wing-folding pattern (23). At present it seems easier to suppose them both to have come from a hypothetical common ancestor than to derive either from any known type of the other. Direct fossil evidence takes the Curculionoidea back to the Upper Jurassic, and it seems likely that the Chrysomeloidea are at least this old, for the first radiation of the Cucujiformia could hardly have occurred later than the beginning of the Jurassic period.

The relationships of the "lower" cucujiform superfamilies Cleroidea, Lymexyloidea, and Cucujoidea are unclear. One might see an analogy between the adaptive radiation of early Cucujiformia and that of proto-Coleoptera, the Cleroidea representing the predacious line (cf. Adephaga), the Lymexyloidea representing the wood-borers (cf. Archostemata), and the Cucujoidea approximating the original (mould-eating) habits (cf. Myxophaga-Polyphaga). The assumption that Cleroidea represent an originally carnivorous stock may seem questionable in view of the fungivorous habits of such primitive existing types as *Phloeophilus* and Ostominae. The Chrysomeloid-curculionoid stock might, on this theory, be a later offshoot of the cucujoid stock, sharing a special mode of adult mid-gut formation in the pupa [Mansour (45)].

Within the Cleroidea, there seems to have been an early bifurcation of the family tree, one branch giving rise to the Melyridae (with Phycosecidae) and the other to Trogositidae (with *Phloeophilus*). A further development from the trogositid stock may have given rise to Chaetosomatidae (13) and Cleridae. Within the Trogositidae, differences in the larval and adult mouth parts seem to be correlated with differences in food: in fungivorous forms the larvae have a distinct maxillary articulating area and the mala bears heavy spines, whereas in the adult the mandible has a distinct molar part; in the carnivorous types the larval maxillae have the articulating area obsolete and the mala bears comparatively fine hairs, and the adult mandibles lack a molar part. Beyond this, it is difficult to find any adaptive explanation of the key changes in cleroid phylogeny. The mandibles of primitive adult trogositids (e.g., *Diontolobus*) are much like those of primitive cucujoids, both adult and larval. This is another possible instance of the persistence in adult beetles of mouth part characters older than those of their larvae. The adult *Hendecatomus*, whose mouth parts much resemble those of lower dermestoids, offers a probable parallel in Bostrychoidea.

Superfamily Cucujoidea.—Though excelled by Chrysomeloidea and Curculionoidea in number of species, the cucujoids include more families and a greater adaptive diversity than any other Coleopterous superfamily. It is most easily defined as comprising the residue of Cucujiformia after the comparatively well-marked groups Cleroidea, Lymexyloidea, Chrysomeloidea, and Curculionoidea have been split off from it. In view of this unsatisfactory

definition and of the diversity of the included forms, it is natural to suspect the group of being worthy of subdivision into two or more superfamilies. This suspicion is likely to be strengthened when it is recalled that Cucujoidea include the Heteromera and Clavicornia (*s. str.*), widely separated in the older classifications and brought together for the first time by Böving & Craighead (3). It is, in fact, possible to make a broad division of Cucujoidea, both adult and larval, corresponding roughly with the old Clavicornia-Heteromera division (13). Characters which can be used for this purpose include the tarsi, aedeagus, and wing venation in the adults, and the mandibles, maxillae, and tenth abdominal segment of the larvae. If the 5-5-4 tarsi are relied on, many forms which are Heteromera in other respects but have 4-4-4 tarsi (e.g., colydiids) will be excluded; if the sheathlike or pseudo-trilobe aedeagus is relied on, forms with 5-5-5 tarsi in both sexes (e.g., Byturidae, Biphyllidae) will be included in Heteromera. The basic wing-venational pattern of Heteromera is closely approached in such Clavicornia as Erotylidae. The absence of the prostheca to the larval mandible would place *Byturus* in Heteromera leaving *Biphyllus* in Clavicornia, in which various Nitidulids and Phalacrids also lack the prostheca. The blunt maxillary mala is found in various Clavicornia (Sphindidae, Erotylidae, Endomychidae) as well as in Heteromera. In typical Clavicornia, the larval tenth abdominal segment is rather pygopodlike, short conical, or cylindrical and is situated postero-ventrally; usually it is either entirely membranous or evenly sclerotized all round. In typical Heteromera this segment is not at all pygopodlike, and is situated more ventrally, transverse, and with somewhat sclerotized dorsal and ventral lips. *Byturus* and *Biphyllus* have the clavicorn type of structure, as have some undoubted Heteromera like *Tetratoma*. If the Clavicornia-Heteromera division is to be employed, I think the phylogenetically soundest procedure might be to base it essentially on the structure of the aedeagus and include Byturidae and Biphyllidae in Heteromera as well as Cisidae, Mycetophagidae, Colydiidae (excluding Cerylonidae), etc. The interrelationships of the Heteromeran families are complex and can hardly be usefully discussed in the present state of our knowledge.

Within the Clavicornia, there is at least one apparent phylogenetic subgroup which can be distinguished at present—that of the families allied to endomychidae. These include the Cerylonidae (13), Endomychidae, Corylophidae, Coccinellidae, Discolomidae, Merophysiidae (13), and Lathridiidae. They are set apart from other Clavicornia by a 4-4-4 (or 3-3-3) tarsal formula and by a peculiar type of wing venation (23), and their various larval types are connected by transitional forms. Böving & Craighead (3) assume that the sharp-pointed maxillary mala of rhizophagid and similar larvae is a primitive feature, and it certainly tends to coexist with a type of mandible very like that of lower dermestoids and, hence, probably also is primitive. At present it seems uncertain whether the primitive larval spiracles of Cucujoidea were bicameral as in rhizophagids, nitidulids, etc., or annuliform as in Sphindidae and most Cucujidae; our understanding of

coleopterous phylogeny generally would be greatly advanced by the discovery of the adaptive significance of these different spiracular types. In the adults, 5-5-4 tarsi in the male sex might well be an ancestral feature in Cucujoidea, likewise the presence of parameres articulated to the aedeagus, though these are lacking in such primitive forms as Sphindidae and *Ericmodes* (*Protocucujus* Crowson) (13), as well as in Cleroidea and Chrysomeloidea-Curculionoidea.

Superfamily Chrysomeloidea.—Our understanding of phylogeny in Chrysomeloidea suffers from the lack of useful early fossils. As far as I am aware, no clearly chrysomeloid types have been found as fossils in pre-Tertiary deposits. According to the general characters, diversity, and relationships of the group, it must certainly go back to the early Cretaceous era, and probably to the Jurassic, as does Curculionoidea. The early forms would probably have had the characters of Cerambycidae (*s. lato*). In existing forms, the most primitive types of wing venation occur in cerambycids, in Philinae, many Aseminae, and Lepturinae much resembling primitive cucujoids (e.g., *Ericmodes*). On the other hand, the presence of a distinct gula in cerambycid larvae (except *Distenia*) seems likely to be derivative in comparison with its absence in chrysomelids, bruchids, curculionoids, and most cucujoids. However, the presence of a distinct ligula may be primitive in cerambycid larvae, and it is tempting to regard the urogomphi of Aseminae larvae as primitive likewise; if urogomphi are found in the as yet unknown larva of Philinae, it will support the theory that early Chrysomeloidea had these cerambycid characters. Provisionally, we may suggest that the ancestral chrysomeloid larva lived internally in some sort of stem, probably a dead woody one, and that the adult was free-living, active, and short-lived, probably frequenting the Jurassic precursors of flowers. It had departed from the clavicorn habitus much as various Heteromera with similar adult habits have done, e.g. Oedeмерidae.

Within the Cerambycidae, phylogenetic relationships are by no means evident or generally agreed. It is gradually becoming accepted that the enormous group Lamiinae is derivable from ancestors in or near Aseminae, perhaps near the existing Atimiini (9, 57). Some of the peculiarities of adult lamiines may be related to the habit of chewing actively into bark, either for feeding or preparatory to oviposition. It also seems that the old Prionidae (Prioninae, Anoploderminae, Parandrinae) form a more or less natural grouping, but the position of Disteniinae is as problematic today as it seemed to Böving & Craighead (3) and Forbes (23). An elaborate family tree of cerambycids, based on wing-venation and constructed from existing forms only, was published by Saalas (57); the surprising thing about this diagram is that, for all the unsoundness of its ostensible basis, it does suggest the main outlines of cerambycid evolution. Lameere (43a) suggested that the existing genus *Parandra* had formerly included the ancestors of the entire family Cerambycidae; many characters, both larval and imaginal, of *Parandra* are inconsistent with this suggestion, notably the wing venation (57), metendo-

sternite (11), ovipositor, and maxillae of the adult, and the ventral head structure and absence of ocelli of the larva (9). Gahan (26) advanced the much more plausible suggestion that Philinae are a primitive group among existing cerambycids. From a Philinae-like ancestor we can imagine the derivation of Prioninae by way of forms like *Anoeme* (15), of Anoploderminae via forms like *Pathocerus*, while *Erichsonia* and *Parandra* might well come from anoplodermine ancestors. Aseminae and Lepturinae might be direct offshoots of Philinae. The Cerambycinae may be related to Lepturinae much as Lamiinae are to Aseminae. Forms of rather doubtful position of this theory include Disteniinae, Oxypeltini, and Vesperini.

Within the Chrysomelidae (*s. lato*, including Bruchidae), there are a number of clearly defined major groupings with interrelationships which pose problems as difficult as those created by the interrelationships of Cerambycidae. Phylogeny within the family has been discussed by Chen (7), Chûjô (8), and Monros (48). The larval characters, and to some extent those of the adults, clearly establish the affinity of Bruchidae and Sagrinae, inasmuch that it becomes difficult to justify familial separation between them. In many respects the bruchids would appear to be the more derivative group of the two, and it is easy to imagine their larval habits arising as a specialization from those of Sagrinae. If, as would be reasonable, we take resemblances to cerambycids as indicators of primitiveness in chrysomelids, then we shall be led to regard either Megalopodinae or Orsodacninae as the most primitive group in the family. The megalopodines, alone among chrysomelids, have the mesonotal stridulatory file characteristic of cerambycids; the aedeagus in megalopodines and *Orsodacne* has the bilobed tegmen and long median struts which are usual in cerambycids (7). Megalopodine larvae (48a) are perhaps more cerambycid-like than any others in Chrysomelidae.

There are reasons for postulating a direct link between Megalopodinae and the large division Camptosomata (Megascelinae, Clytrinae, Cryptocephalinae, Chlamisinae, Lamprosominae) in which the larvae have become free-living within the shelter of an excrementitious case. Megalopodine larvae are stem-borers or leaf-miners (*Zeugophora*) with a prognathous head having a pronounced endocarina and no distinct epicranial suture, whereas in Camptosomata the larval head is markedly deflexed, the endocarina lacking, and a well-marked epicranial suture present. It seems possible that these camptosomatan features are derivative in relation to the megalopodine ones, somewhat similar relations occurring between larvae of Anobiidae and Bostrychidae, or Apionidae and Attelabidae (*vide infra*). The imaginal features of Camptosomata—laterally bordered prothorax, entire ligula, apically two-toothed mandibles, reduced tegmen of the aedeagus—parallel those of other "higher" Chrysomelids. These higher Chrysomelid characters are seemingly polyphyletic, having been developed also in the Aulacoscelinae-Chrysomelinae and Eumolpinae-Galerucinae lines. If the same set of four apparently independent changes has taken place in at least three different lines, it strongly suggests some common adaptive basis. I think it probable that

ancestral adult chrysomelids did not eat foliage or any solid plant tissues, but probably like *Orsodacne* (or Cerambycidae-Lepturinae) frequented flowers or possibly cut plant shoots to feed on exuding sap like Megalopodinae. The changes in the adult mouth parts may be adaptive to eating solid plant tissues, that of the prothorax (and probably of the general body form) to the habit of sitting on leaves, and that of the aedeagus to a consequent change in the mode of copulation. In the higher chrysomelids there is also a marked tendency to develop a large closed cell in the wings, rather like the wedge cell of primitive cerambycids, but here likely to be a secondary development, combined with only three or four anal veins in the main group (12, 22), whereas cerambycids (57) with a wedge cell always have five; this development in the anal region I suspect may be connected with the assumption of a short, broad, convex body form, the primitive types having comparatively long and narrow elytra and abdomen.

Superfamily Curculionoidea.—The possession of a free labrum, of normal flexible maxillary palpi, and of a distinct lacinia [Ting (66)] are almost unquestionably primitive features in adult curculionoids; all three characters seem to be universal in, and confined to, the families Anthribidae and Nemonychidae, within this superfamily. The anthribids have universally been recognized as a distinct family since the days of Lacordaire (42), but the Nemonychidae (*Rhinomacerini* auctt.) have had a far more chequered history. They were already associated with Anthribidae on the correct grounds by Redtenbacher (54), but the influence of Lacordaire, who attributed far less importance to the mouth parts generally than previous authors had done, rapidly became predominant and nearly all subsequent workers followed him in associating nemonychids with Rhynchitinae [one exception was Kolbe (40)]; this tradition has persisted up to the present day in the work of Voss (67, 68). As soon as larvae of nemonychids became known, it was evident that they were much closer to those of Anthribidae than any other curculionoid type (1, 17), and that they were widely unlike those of Rhynchitinae. The main question now at issue is: can Nemonychidae be retained as a family distinct from Anthribidae? In the imagines, the characters associating nemonychids with anthribids seem to be entirely primitive; they were presumably present in ancestral curculionoids. The larval similarities may also be primitive, though at present this looks far less certain. If all common characters of the two families are primitive, then there are no good grounds for a special association of them in phylogenetic classification; the nemonychids might well be closer to the ancestors of the rest of Curculionoidea than they are to anthribids. In this connection we may note that the oldest (Jurassic) known fossil Curculionoids show a long straight rostrum resembling that of *Cimberis* (*Rhinomacer* auctt.) rather than of any known anthribid. If the ancestral curculionoids had characters very like nemonychids, we might derive, on one line, the Anthribids (including *Urodon* = *Bruchela*) by a change which initially affected the body characters much more than the mouth parts, and, on another line, the main curculionoid

stem by a change affecting the mouth parts much more than the body form.

Setting apart the anthribid-nemonymid group, the remaining curculionoids can again be divided by the condition of the gular sutures. In the Belidae, Oxycorynidae and Proterhinidae (= Aglycyderidae), they are separate as in nemonymids, and in all the remaining curculionoids (including *Allocorynus*), the gular sutures are fused. The small groups set apart in this way are also related by their larval, and several additional imaginal, characters; their present-day distribution and the occurrence as fossils of at least oxycorynids in the Baltic Amber and the North American Eocene suggest a relict character. Kuschel (in litt.) has suggested that they might all be united in a single family. The genus *Allocorynus* is a possible link between this group of families and the rest of Curculionoidea.

After removal of the anthribid and belid groups, indications of yet another phylogenetic bifurcation can be discerned in the remaining curculionoids, the indicators this time being the maxillary palpi of the adults and the larval head. In the Attelabidae (including Rhynchitinae, Pterocolinae, etc.) the palpi are four-segmented as in belids, etc., and the larval head is retracted; in all the rest, the palpi have three or fewer segments and the larval head is normally exerted. *Allocorynus* once again occupies a rather intermediate position and logically seems entitled to an independent family.

This bifurcating phylogenetic pattern can be traced even further, for after setting aside the attelabids, the remaining curculionoids can be divided into two, this time by a larval character—the condition of the frontal sutures. In the Apionidae (13) and Brenthidae, these frontal sutures extend extend right to the front margin of the head capsule as in Attelabids while in Curculionidae s. str., also in Scolytidae and Platypodidae, they are closed in front by narrow sclerotized bars (17). The phylogenetic implications of this have not been generally recognized. It has been customary to treat brenthids as a very isolated group, only remotely related to other curculionoids, and, as an indication of this, emphasis has usually been laid on the two-segmented legs of brenthid larvae. Similar legs occur in many anthribid larvae but not, as far as known, in nemonymids, in the belid group, nor in attelabids. If these legs are an ancestral curculionoid character, we shall have to postulate a very polyphyletic loss of them; to me it seems more probable that they are secondarily derived, at least in brenthids, from projecting pedal lobes such as occur in many Apionidae. The larvae of Eurhynchini (excluding *Cylas*), from which brenthids might be derived, are unknown. The position of the genus *Ithycerus* is likely to remain doubtfully between Apionidae and Curculionidae until its larva is discovered. Most authors have laid heavy stress on the condition of the adult antennae as a classificatory character at this point; in all the "lower" curculionoids (nemonymids, anthribids, belids, oxycorynids, proterhinids, attelabids, and *Allocorynus*), the antennae show no sign of geniculation, the scape is little longer than succeeding segments, also the club is loose and three-segmented. In contrast with this "orthocerous" condition, the antennae of most curculionids, scolytids, and platy-

podids are geniculate with a long scape and a compact club—the “gonatocercous” condition. In brenthids the antennae are decidedly orthocercous, as also in Eurhynchini; in Nanophyini they are clearly gonatocercous, in Apionini more or less intermediate (as also in *Ithycerus*). Classifications relying mainly on the antennae have placed Nanophyini in the Curculionidae *s. str.*, though the *Nanophyes* larva is typically apionid (17). In fact it is difficult to frame a key couplet to separate adult Apionidae from Curculionidae.

So far, at each stage a small group has been split off from the main curculionoid trunk by the possession of some primitive character; when we come to deal with the great residual mass of Curculionidae it is tempting to look for a repetition of this pattern. Two characters have been suggested which might offer a possibility of this: the condition of the tergal folds of the larval abdomen (17), and the aedeagus of the adults (5). The larval abdominal segments in various small groups (Rhynchaenini, *Mecinus*, *Gymnetron*, *Stenopelmus*, *Orobitis*) show only two tergal folds like most apionids, instead of three as in normal curculionids (and Brenthidae). In the aedeagus, the Erirrhini *s. str.* (5) alone show a complete cucujoid tegmen similar to those of apionids, attelabids, *Ithycerus*, and others. These two characters are quite discrepant in their indications and can hardly both be primitive.

The time-honoured divisions of Scolytidae [Scolytoidea; Schedl (59)] and Platypodidae are convenient and easily recognized in the adults, though no familial larval characters for scolytids have yet been found. The fossil scolytids of the Baltic amber have been critically revised by Schedl (58), who showed that all the authentic specimens represented very primitive types of the family, mostly Hylastinae. This evidence indicates that the group was at an early stage of its evolution at the beginning of the Oligocene, and probably did not originate before the Eocene, in which period unmistakable fossil curculionids occur. Old records of *Platypus* in Baltic amber were not substantiated by Schedl; it would appear that collections of Baltic amber fossils have been liable to contamination with specimens in more recent fossil resins, perhaps even by deliberately faked inclusions of modern insects in amber-like resins. The matrix of all type material should be subjected to critical tests, both for succinic acid and for the possibility of faking.

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NORTHERN BITING FLIES¹

By B. HOCKING

Department of Entomology, University of Alberta,
Edmonton, Alberta, Canada

Across the northern regions of the Old World and the New, on the tundra and in the boreal forest where snow lies regularly throughout the winter, enormous populations of insects in some families of the orthorrhaphous Diptera, the Heleidae, Culicidae, Simuliidae, and Tabanidae develop during the short summer season. These groups are systematically close and linked by peculiar similarities of habits, among which man tends to regard that of feeding on his blood before developing eggs as the most important. Other flies which feed on blood occur in these areas, and purists may object that these flies suck rather than bite, but my title has been used as it stands by most of those who have endured, and yet enjoyed, extensive field investigations into these populations in the New World over the last twelve years. In the Old World, the Russian term *gnus* is roughly equivalent to "biting flies," although it apparently includes *Stomoxys* and *Phlebotomus*.

The southern limit of these fly populations is set roughly by the 5°F. mean January isotherm; the northern limits surge back and forth with the seasons. I have tried to cover this field of northern biting flies from the most recent previous reviews to early 1959. Work on related groups in other areas is referred to if it illuminates problems in this area.

GENERAL AND BIBLIOGRAPHICAL

The Arctic Institute of North America's Arctic Bibliography (267), which has strong biological emphasis, and Stage & Sollers' "References to Literature of Interest to Mosquito Workers and Malariologists," appearing in each issue of *Mosquito News*, are both valuable. A good account of the climate of the Canadian part of the New World area is given by Kendrew & Currie (122) and of the topography from the insect's viewpoint by Munroe (176). The topography of the Old World area is treated by Suslov (260), and the climate by Alisov (1). Nuttinson (185) compared the agricultural climatology of Siberia with that of North America, and an excellent Old World view of New World climates is given by Vitvitskii (270). Other papers with good bibliographies of the special fields with which they deal are listed in the bibliography of this review (1, 6, 11, 12, 34, 35, 40, 41, 48, 51, 52, 57, 58, 59, 60, 61, 62, 70, 87, 94, 97, 98, 100, 104, 108, 109, 128, 142, 151, 152, 153, 154, 155, 159, 160, 161, 171, 172, 176, 178, 180, 192, 194, 200, 208, 217, 219, 220, 221, 227, 239, 252, 257, 267, 269, 286).

MORPHOLOGY, SYSTEMATICS, AND EVOLUTION

Morphology has been an unfashionable field; there has been correspondingly little advance. Textbooks still appear with virtually nothing added

¹ The survey of the literature pertaining to this review was concluded in July, 1959.

to the descriptions by Giles (81) of the structure of mosquito larvae. New information has largely resulted from the search for new systematic characters, sorely needed in some groups, rather than from comparative studies in functional or evolutionary morphology [e.g., Knight & Chamberlain (140); Vockeroth (272)]. Hoyt's (109) study of the maxillae and labium in many dipterous families, including the four with which we are concerned in this review, and in other orders possibly close to their precursors, remains a valuable contribution when divested of its specious evolutionary argument. Smart's observations on the coxo-subalar muscle also have evolutionary significance (249).

Surprisingly, the most striking morphological advances have been made in the immature stages of the smallest insects, the heleids; however, there was most room for advance here. Furthermore, much of the advancement has been in association with systematic work [Kettle & Lawson (128, 129); Lawson (148); Nielsen (181)]. Megahed (161) studied the morphology of the alimentary system of *Culicoides*. The taxonomy of the Old World Heleidae still rests largely on the work of Goetghebuer & Lenz (83), although the systematic work mentioned above and studies from Russia [Amosova (2); Dzhabarov (63); Gutsevich (84, 86); Gutsevich & Dzhabarov (90)] and from the New World [Downes (57, 61); Ewen & Saunders (66); Foote & Pratt (68); Saunders (232); Wirth (284)] must modify their conclusions. Studies, such as that of Khalaf on speciation in *Culicoides* (132), might well be accorded other genera.

In the mosquitoes, the sense organs have received most attention. Thanks to the work of Sato (227 to 231) we now begin to know something of the fine structure of the mosquito compound eye; this opens up great possibilities for relating structure with function, and, thence, with behavior. Stewards' (255) work will shortly put us in a similar position in respect to the antennal organs. Day (49) described what he believes to be a pressure sensitive organ. Clements (36) described the antennal pulsating organs in mosquitoes and related Nematocera, and Belkin (17) abdominal chaetotaxy in the larvae and pupae.

Shtakel'berg's treatment of adults in the *Fauna of the U.S.S.R.* (246) is still used as an authority on the Russian mosquitoes. Monchadskii's work on the larvae of the Russian mosquitoes appeared as a second enlarged and revised edition in 1951 (172); it is interesting to note that, while much new material has been added, the article in the 1936 edition on the evolution of the group has been omitted. Natvig's (178) treatment of the Scandinavian fauna extends into biology and economics. In the New World, Carpenter & LaCasse (34) and local treatments such as Barr (6), Owen & Gerhardt (194), and Rempel (215) have largely supplanted Matheson (159). Mosquito workers the world over welcome the synoptic catalogue of Stone, Knight & Starcke (258).

The new edition of Rubtsov's work on the Russian black flies (223) is the most comprehensive Old World treatment, though not the most con-

servative; this is in contrast with Shewell's (244) arrangement from the New World. Stone holds different views again (Annual Meeting, Entomological Society of America, 1958). A number of local lists with some taxonomic revision have appeared [Nicholson & Mickel (180); B. V. Peterson (199); Sommerman (253); Stone (256); Stone & Jamnback (257)]. The composite nature of many species groups is becoming evident (42, 261).

Some of these papers contain morphological contributions; Lewis (151) published a developmental study of the structure of *Simulium damnosum* Theobald. A study of the fine structure of the larval mouth brushes in relation to their function is needed, and surely some uses other than those made of them by systematists can be found for the pedisulcus and the calcupala on the hind leg of the adult.

In the tabanids, the most important contribution in gross morphology is that of Bonhag (21, 22) on *Tabanus sulcifrons* Macquart. Scudder's (235) histological study of the sense organs extends into function and parallels in some respects that of Steward on mosquitoes.

Mackerras (152 to 155) in a comprehensive evolutionary and zoogeographical systematic treatment of the tabanids set an example which might well be followed in other groups and in other countries. The tabanid volume of the *Fauna of the U.S.S.R.* by Olsuf'ev appeared in 1937 (188). Kauri (121) contributed to our knowledge of the Scandinavian fauna. Philip (203) took one of the few steps toward the sadly needed coordination between workers in the Old World and the New; it is a pity he could not have gone further.

The Canadian Northern Insect Survey (70), the American Alaska Insect Control Project (265), and other parties have contributed innumerable specimens to the great collections. This had led to some studies in the open literature (137, 243, 245, 256, 273, 274, 275) and to a number of distribution maps limited to the programs in question and restricted in circulation. However, the great bulk of this material, in Canada at least, has not yet borne fruit on public trees; it is to be hoped that this will not be long delayed. Much work remains to be done on these groups in the field; a New World version of Shtakel'berg's (adults and pupae) and Zimin's (larvae) keys to the "synanthropic" flies (247, 290)² would be convenient.

Modern procedures in systematics are abundantly and well represented among the biting flies; if the excellent chromosome studies of Rothfels & Dunbar (220) with black flies could be extended into the other groups, many problems might be solved. Hovanitz' observations on the genetics of speciation in mosquitoes (108) were followed by critical studies by Knight (138, 139), Knight & Malek (141), and Mattingley *et al.* (160) on hybridization and speciation in the *Culex pipiens* Linnaeus group and by other studies, reviewed by Rozeboom & Kitzmiller (221), on sibling species in mosquitoes.

² Since this manuscript was written I have received a copy of a book (16a), prepared by ten Russian workers under the direction of Dr. W. N. Beklemishev, which appears to supersede these publications. Keys to all Annulata of medical importance in Russia are included, with 84 pages of illustrations.

Micks & Benedict (165) and Micks & Partridge (167) with infrared spectrophotometry, Micks (164) and Micks & Gibson (166) with chromatography, Downes (57) with food habits, Horsfall & Craig (106) with eggs, Bohart (19) with first instar larvae, and Darsie (38) with pupae have contributed to systematics or to phylogeny at various levels. Traditional contributions continue [van Emden (64)].

But when all this is told, there remains a crying need for thorough comparative studies of the morphology of particular organs and organ systems in all of these groups. Snodgrass (*in litt.*, 1959) recently drew my attention, for example, to the peculiarities of the wing articulation in mosquitoes, which he finds quite different from that of all other insects except some related Nematocera. This echoes strangely a plea for a study of these structures by Rodendorf in one of the few recent papers on the evolution of the nematocerosous Diptera from a palaeontological viewpoint (217). And, speaking of fossils, surely North American Nematocera have been fossilized, too? Such studies will pay dividends in taxonomy, and through physiology and ecology, in control.

PHYSIOLOGY AND BEHAVIOR

Largely because of their resistance to laboratory culture, northern species have been little used in physiological studies, and most of our knowledge of them is by inference from other species.

Some physiological data, and rather more behavioral data, are to be found in systematic treatments of mosquitoes and black flies. There has been less work done on the physiology of the heleids and tabanids. The heleids are inconveniently small. Becker (15) was able to show that the light reactions of larvae of *Culicoides circumscriptus* Kieffer are dependent on their nutritional state, and starved larvae become photopositive. The tabanids are difficult to rear, but young adults are readily caught in the field and give a useful term of laboratory life; they are large and rugged animals and tolerate all kinds of laboratory mayhem.

The physiology of mosquitoes was reviewed in 1949 by Wigglesworth (281). Progress has been made in elucidating the nutritional requirements of both larval and adult stages [Dimond *et al.* (53); Galun & Fraenkel (78); Singh & Brown (248)] and at least some of the required feeding stimuli [Hosoi (107), Lang & Wallis (143)]. Nutritional stimuli and requirements, as well as studies of feeding mechanisms [Day (48), Denisova (50), O'Rourke (191)], show so much variation between species that studies of this sort with northern species would certainly not be redundant. The respiration of larvae of *Anopheles quadrimaculatus* Say has been studied by Jones (118), and of adults of several species by Mercado, Trembley & Brand (163).

The dependence of ovarian development on a hormone was demonstrated by Clements (35) in *Culex molestus* Forskål and *Anopheles stephensi* Liston and beautifully confirmed and associated with the corpora allata in transplantation experiments by Larsen (145). Gillett (82) found that the release

of the hormone is influenced by adult feeding, in particular fruit juice as against blood in *Aedes aegypti* (Linnaeus). Lea *et al.* (149) obtained egg production on a milk diet. Schuh (234) reported the inhibition of metamorphic changes in the hind gut of *C. pipiens* by colchicine. The apparent disagreement between Beckel (13) and myself (99) regarding the autolysis of flight muscles in *Aedes communis* (De Geer) may reflect the *C. pipiens*-*C. molestus* situation or may be more simply explained as a difference between laboratory and field conditions.

Since the difficulty in securing mating is often a major block in the rearing of northern species, Burcham's (32) report of an artificial insemination technique for *A. aegypti* is timely. Beckel (14) found that *Aedes hexodontus* Dyar and *A. aegypti* lay eggs on rough, dark surfaces in preference to smooth, light ones, but rather surprisingly that *Aedes campestris* Dyar & Knab chooses open water in preference to moist filter paper. Borg & Horsfall (23) and Horsfall (105) demonstrated the dependence of hatching on low oxygen tension and, hence, on the presence of living bacteria in *A. aegypti*, *Aedes vexans* Meigen and in *Psorophora confinnis* (Lynch Arribalzaga).

Hodgson (102) reviewed chemoreception in arthropods, and Dethier (52) the sensory physiology of blood-sucking species. A bibliography with abstracts dealing with smell in insects and applications of such knowledge, with some emphasis on biting flies, appeared recently (100). The physiology of sense organs is still inadequately supported by morphology. Chemoreception in relation to oviposition has been studied by Wallis (278) in ten species and by Hudson (110) in two of the same species; they agree in localizing salt sensitivity on all tarsal articles. Frings & Hamrum (71) found contact chemoreceptors also on the labella but not on the palpi or antennae; Day (48) found them within the buccal cavity.

Roth (218) and Rahm (211) investigated the receptors involved in host-seeking behavior in *A. aegypti*, Roth also in *A. quadrimaculatus*; both found the antennae important in attraction from a distance, the tarsi and palpi at close range. Roth & Willis (219) and Willis & Roth (283), respectively, showed hygro-sensitivity and sensitivity to carbon dioxide in the antennae of both sexes of *A. aegypti*, and Reeves (212) reported specific differences in attraction to carbon dioxide in the field. An observation by Nielsen (182)—flash bulbs will disturb nesting mosquitoes, while electronic flash will not—challenges explanation and suggests new techniques for the study of nocturnal and crepuscular species.

Omardeen (189) found that the larvae of *A. aegypti* preferred a temperature of 23–32° C. in the second instar but 28–32°C. in the third and fourth instars. He also found that negative phototaxis first developed in the fourth instar and became intense in the pupae; he attributed this to the developing imaginal compound eyes.

Arnal (5), Bässler (11) in a general study of the significance of Johnston's organ in orientation, and Wishart & Riordan (285), using *A. aegypti* in one of the few quantitative studies of responses to sounds in insects, have

marked the centenary of Johnston's description of the organ bearing his name (117).

Less is known of the physiology of black flies. Much attention has been given to blood-feeding and its relationship with mating and ovarian development [D. M. Davies & B. V. Peterson (41)]. Lewis Davies (45, 47) put the emphasis on age in relation to these phenomena, Rubtsov (224) on nutrition, and Ogata (187) on diurnal rhythms. Lewis Davies (44) reported *Prosimulium ursinum* Edwards in Norway to be fully gravid on emergence from the pupa, non-biting, and probably parthenogenetic. Downe (54) used precipitin tests to study the rate of digestion of blood meals. Hinton (95) studied the larval chromatocytes and their function at metamorphosis and the origin and development of the indirect flight muscles in *Simulium ornatum* Meigen (96).

Frings & O'Neal (72) found contact chemoreceptors on the labella and tarsi of *T. sulcifrons* and measured median acceptance thresholds for sugars and rejection thresholds for various electrolytes.

BIOLOGY AND ECOLOGY

The biology and ecology of the Canadian biting flies have been reviewed by Freeman & Twinn (70) and Twinn (268, 269). Pavlovsky, Pervomaisky & Chagin (197) and Gutsevich (87) performed a similar service for the Old World. These reviewers and many other authors on ecology have treated control as well, and many of the more general papers [Frohne & Sleeper (77); Gutsevich (88); Kettle (127); Montschadsky & Radzivilovsky (174) cut across family subdivisions.

General papers on the ecology of the blood-feeding heleids in Russia have been published by Gutsevich (85, 89), Molev (169), and Remm (213). Kettle (126) found a close relationship to exist between the rainfall during adult activity and the larval populations in the following generation of *Culicoides impunctatus* Goetghebuer. Downes (58) studied the swarming flight and the mating behavior of *Culicoides* and of the biting nematocera generally (60). He considered it likely that swarming functions as an isolating mechanism and further that, since each individual responds to the marker independently, the swarm is not primarily an example of gregarious behavior. This may be true for swarm formation, but surely does not apply to the maintenance of the swarm [cf. Haskell (91)]. Kettle (125) found the sex ratio to be normal in 26 British species of *Culicoides*. Fallis & Wood (67) demonstrated, experimentally, that an ornithophilic species of *Culicoides* is a suitable intermediate host for a *Haemoproteus* of ducks, and Molev (168) reported on *Culicoides* in relation to disease transmission in horses.

Mosquito ecology has been reviewed by Bates (12), and Horsfall compiled data on bionomics and distribution (104). General papers on mosquitoes include those of Frohne on *Culiseta impatiens* (Walker) in Alaska (73) and on zonal distribution in relation to life cycles (76). Jenkins & Knight (115)

reported the presence of 27 species with their associations and habitat types at southern James Bay in Canada. Remm (214) reported on a similar study in Russia.

Frohne (74) studied the salt marsh habitat in Alaska where *Aedes punctodes* Dyar is the dominant species; this species tolerated chlorides at one part in 200. Rumsh (225) divided 20 Arctic species of Europe and Asia into groups characteristic of the tundra, forest tundra, taiga, forest steppe, and steppe.

Haufe & Burgess (94) studied the effects of temperature on larval development of *Aedes impiger* (Walker), *Aedes nigripes* Zetterstedt, *Aedes punctor* (Kirby), *A. hexodontus*, *Aedes excrucians* (Walker), and *A. communis* and obtained values for threshold of development temperatures and degree-days to emergence, thus permitting prediction of emergence dates from temperature records. Haufe (93) also studied the behavior of larval *A. communis* in relation to temperature and insolation and of adult *A. aegypti* in flight (92) in relation to atmospheric pressure. Snow and Pickard (251) found that vertical components in the flight of *Mansonia perturbans* (Walker) are favored by changes in light intensity at about the 1 ft. c. level. Platt *et al.* (206) correlated the behavior of *A. quadrimaculatus* toward moisture, temperature, and light in the laboratory with field activity. Larsen (144) found a diurnal rhythm of activity in *Aedes* spp. with peaks at lowest wind speed and highest humidity; these factors were more important than temperature. Khelevin (134) found in eggs of *Aedes caspius dorsalis* (Meigen) a summer diapause as an adaptation to dry conditions and winter diapause as an adaptation to cold.

Despite the very evident importance of population density in the ecology of biting flies, little attention has been given it. Wallis (279) found that the population density of ovipositing females of *A. aegypti* had little influence on the acceptable salinity of water for oviposition except that dense populations would lay some eggs at higher salinities. Terzian and Stahler (262) found that adults of *A. quadrimaculatus* from crowded larvae were smaller and lighter, died sooner, and showed lower biting rates than those from normal larval population densities. Bar-zeev (8) on the other hand, found that if abundant food were provided, population density had no effect on growth rate or mortality; larval food shortage in his experiments resulted in small adults, which took less blood and developed fewer eggs. Surtees (259) traced the variations in adult populations of a West African strain of *A. aegypti* to variations in larval population density in clay water pots, where high populations delayed development and caused starvation mortality.

Important advances have been made in our knowledge of the relationship between mosquitoes and their hosts. The relative significance of odor, contact chemical stimuli, thermal stimuli, and mechanical stimuli in host finding is being unravelled as the result of studies by Rahm (208, 209), Schaefferberg & Kupka (233), Brown alone (26) and together with Burgess

(33), Smart (250), and Thompson (264), and Laarman (142). Internal factors in the mosquito influencing this relationship have been investigated by Lavoipierre (147) in the laboratory and by Montschadsky (171) in the field. Internal factors in the host have been studied by Kingscote & Francis (136), Rahm (210), and Khan (133), and the behavior of the host has been studied by Breev (24). The problem has also been approached by observing in the field the end results in host selection and host preferences. Markovich (158) reported the overwhelming abundance of *Anopheles maculipennis* Meigen in goat and cow sheds as compared with houses, Wallis (280) reported *Culiseta morsitans* (Theobald) feeding on pheasants, Downe & West (56) established host preferences for many species by precipitin tests.

A few reports of parasitism or, at least, phoresy by protozoa and algae (115), fungi (6, 240), and mermithids (116) and of predation by *Mochlonyx* (112) and by a stickleback (74) have appeared. The stickleback is considered to be important in natural control.

The relationships of mosquitoes with plants have, naturally enough, received much less attention. Dutilly's bibliography (62) provides a starting point, and Downes (59) has reviewed the feeding relationships and emphasized their importance. This is another field where further study should be profitable.

Since I reviewed dispersal (98), Shemanchuk and others (241, 242) have used radio-phosphorus markings to establish flights of 6.6 miles and 1400 yards by female and male *Aedes flavescens* (Müller), respectively. Quarterman *et al.* (207) have recorded flights up to 1.5 miles by *Psorophora discolor* (Coquillett). Enikolopov (65) has reported flights of 1500 m. by *Anopheles algeriensis* Theobald. Horsfall (103) has reported on a migration of *A. vexans*.

A number of general papers on the ecology of black flies have laid the foundations for more detailed work [Davies (43); Fredeen (69); Ogata (186). B. V. Peterson (200); D. G. Peterson & Wolfe (201); Sommerman *et al.* (254); Usova (269b)]. As yet, the more detailed work has been scarce. Rubtsov (222) attributed variations in intensity of attack to variations in plankton production and, hence, in larval nutrition, Lewis (150) found a relationship between the time of biting and the gonad cycle. Downe & Morrison (55) found, by serological methods, that horses and cattle were fed upon by most of the species studied, and pigs and chickens by few; only one out of 1672 specimens had fed on more than one host species. Muirhead-Thomson (175) reported communal oviposition by *S. damnosum* rather similar to that described for *Simulium venustum* Say (101).

Williams and Davies (282) reported catching numerous male and female black flies in ultraviolet light traps, evidence of surprising nocturnal activity. Phillipson (204, 205) showed that, with *S. ornatum*, current speed was more important than oxygen content in governing larval distribution in a stream. Variation in oxygen content had no influence above 50 per cent saturation. Eighty to 90 cm. per sec. was the preferred current speed. *Simulium monticola* Friedman and *Simulium variegatum* Meigen preferred somewhat higher speeds. Wolfe & Peterson (286) have also studied the influence of current

speed. Yakuba (289) found larval migration to be encouraged by turbidity and rapid rise of water level and to increase with aging.

In 1855, Bold (20) reported a "great abundance of clegs" (*Haematopota*). Wall (277) reviewed sampling methods used for estimating populations of larval tabanids in 1957. Between these two papers there is but little that helps us to understand the size of northern populations. James (111) reported parasitization of mature larvae by *Diglochis occidentalis* (Ashmead), but there seems little evidence that this is of importance in determining population size. It is to be hoped that the recent interest in population dynamics (Andrewartha & Birch (3); Laven (146); Nicholson (179); Solomon (252)) will stimulate further work with these groups.

ECONOMIC IMPORTANCE AND CONTROL

Increasing human population and economic pressure, the interest in the polar route for commerce, and the concern over its possible strategic importance have brought man—increasingly sensitive to minor discomforts—into an irritating proximity to these fly populations. The problem, as it affects man and domestic animals in Alaska, has been discussed by Berg (18); in Scandinavia by Thienemann (263); and in Russia by Beklemishev (16), Montschadsky (173), Pavlovsky (195), and Pavlovsky & Gutsevich (196). At the species level, Frohne discussed *Culiseta alaskaensis* (Ludlow) in relation to man (75), and Lewis Davies *S. ornatum* in relation to cattle (46).

In the complete absence of protection, these fly populations are a direct hazard to life and a considerable threat to sanity. However, it should be noted that, so far as is known at present, under normal conditions the risk of disease transmission is small except near the southern limits of these areas [Crossley (37); Jellison (114); Karpov *et al.* (120); O'Rourke (190, 192)], and protection to provide reasonable comfort (bearing in mind the wide divergence of opinion on this), can easily be provided with existing knowledge. If we add to this, the rapidity and range of dispersal, which demand control operations over very large areas, and the temporary nature of many establishments in the north, it seems clear that on an economic basis alone control operations in the usual sense of killing off populations can rarely be recommended. An exception should perhaps be made of the Heleidae which disperse less vigorously [Kettle (123)]. Nevertheless, there are circumstances in which money is no object and much experimental work on control with insecticides has been done in both the new and the Old World. These same circumstances may demand operations against adults, normally an unsound practice.

Since the review by Twinn (70) North American work has been largely on formulation and application techniques [Brown & Morrison (28, 29); Brown & Mulhern (30); Brown & Watson (31); Jamnback & Wall (113)]. A similar trend is discernible in the Old World [Geffer *et al.* (79); Nabokov & Shlenova (177); Sergiev *et al.* (238)].

The chemical control of *Culicoides* has received far more attention in the Old World than in the New [Kettle (124); Kettle *et al.* (130); Kettle &

Parish (131); Selens (236); Voronin & Molev (276)]. Interest in black fly control has been sustained in the tropics where disease transmission is involved, but little recent work has been done in the Arctic. In Russia in 1956, Usova (269a) obtained good results with DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane] and BHC (1,2,3,4,5,6-hexachlorocyclohexane) in oil but Petrishcheva and Saf'yanova (202) have gone back to emulsions; here, too, the interest has been in application equipment. Sergiev & Nabokov (237) have tried BHC and DDT dusts and BHC smokes as all purpose applications against adult mosquitoes, black flies, and *Culicoides*.

If the large areas and rapid dispersal put insecticidal control at a disadvantage, they are none the less an insurance against the rapid development of resistance problems [Brown, Armstrong & Peterson (27)]. At the same time, we may question whether a little more attention to the possibilities of biological or at least ecological control might not be wise. This question was reviewed for mosquitoes, but without specific reference to northern problems, by Travis (266); Sailer & Lienk (226) studied insect predators on the immature stages of mosquitoes in Alaska; D. M. Davies (40) studied the influence of mites on black fly populations.

There remains the approach to the problem which has the strongest appeal to the biologist, that of personal protection (97). The general question of repellents has been reviewed by Dethier (51) and repellents for biting arthropods by Shambaugh *et al.* (239). Russian contributions to the development of repellents have been minor [Melnikov & Volfson (162); Molev (170); Nudel (184); Vladimirskaia (271)]. There has been most encouraging progress in the more fundamental approach to the development of repellents based on an understanding of the behavior they are desired to disrupt [Bar-zeev & Schmidt (9); Bar-zeev & Smith (10); Kalmus & Hocking (119); Peters & Kemper (198)]. This progress has been sparked, in part, by the alarm over the rate at which insects have been developing resistance to insecticides; the situation offers the prospect of having evolution on our side, favoring for survival strains feeding on the blood of other animals or ultimately, perhaps, autogenous strains.

This new approach, as well as attempts to relate structure to repellency [Roadhouse (216); Geigy & Utzinger (80); Barthel & Gertler (7)], although still without any real understanding of what repellency is, has been forced on us by the increasing difficulty of progress by empirical methods. Wright (287) has put forward a theory of olfaction and the action of repellents which depends on specific infrared absorption. His data lend little support to this, and he confuses perception with reaction. His application of Sutton's theory to the downwind dissemination of odors is a useful contribution (288). Kingscote (135) has sought with more persistence than success for a systemic repellent; this, too, would seem to depend on more fundamental knowledge of what is involved.

Other aspects of personal protection have received less attention; Brown (25) showed that, although fewer flies land on light colored clothing [Davies, D. M. (39) and others], the attack on adjacent exposed skin is not increased,

and Applewhite & Cross (4) that it is not reduced by wearing repellent clothing. Nizovkin *et al.* (183) reported on the combination of repellents with nets. Fly-proof shelters, nets and screens, protective clothing, and protective habits have had almost no attention. The controversy about treatment of bites [O'Rourke & Murnaghan (193), McKiel *et al.* (157)] has had no very useful outcome, but we do know more now about the nature of the reaction [McKiel (156)].

CONCLUSION

To sum up, we may agree that progress made in assessing the problem and developing palliatives is plain; nevertheless, the shakiness of the foundations on which this superstructure rests is almost equally plain. In the systematic field, the foundations are cracked by the Atlantic Ocean and shattered by political and language barriers. Slim reinforcing rods of international congresses and symposia hold the parts precariously together. Less excuse can be offered for the morphological weaknesses; this sort of work usually proves much more exciting than it seems. It needs doing.

The other outstanding problem—a gaping hole on the first floor—is that of population density. It is the size of the populations which constitutes the problem, and we still have no more than a glimmer of an understanding of what lies at the bottom of it. This cannot be solved in a comfortable laboratory outside the area. The solution will prove expensive in time, men, materials, and blood. It will come soonest and most economically where populations are heaviest and insecticides absent.

ACKNOWLEDGMENTS

I am indebted to Dr. G. Zalessky, Moscow, and Dr. W. N. Beklemishev, Institute of Malaria and Medical Parasitology, for much help with the Russian literature, and to many colleagues elsewhere for reprints of their work.

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EVOLUTION AND BIOLOGY OF THE TERMITES¹

BY FRANCES M. WEESNER

800 Colorado Street, Fort Collins, Colorado

In this review of the present status of our knowledge regarding the evolution and biology of the Isoptera, space necessitates certain limitations. Most references are to recent work and many of the papers cited are in themselves reviews. Papers with strictly taxonomic, morphological, or physiological content are not included. A brief synopsis is given of current taxonomic schemes which reflect the relationships and evolutionary trends indicated by morphological characters. Geographical distributions suggest the areas and time of origin of the various groups. Certain aspects of biology, particularly nesting and feeding behavior, indicate many instances of convergent evolution. The reader should bear in mind that the amount of detailed information is small and subject to further investigation and different interpretations.

TAXONOMY

Of the six families of termites, *Uralotermitidae* is known only from Permian fossils. Of the five living families, *Mastotermitidae* has the most primitive characters. These include an anal lobe in the hind wings and an egg mass which resembles the oötheca of the roaches. The termites and roaches probably arose from a common ancestor which had wings and intestinal protozoa [Ahmad (1)]. These microorganisms occur in the wood roach *Cryptocercus* and in the four lower families of termites. *Kalotermitidae* probably arose from a *Mastotermes*-like ancestor, a view which is supported by Ahmad's studies of the imago-worker mandibles (1) and Kirby's studies of the intestinal flagellates (74). *Hodotermitidae* apparently arose independently from a *Mastotermes*-like form. *Rhinotermitidae* is thought to have developed from some early *hodotermitid* which still possessed ocelli. The most highly evolved family, *Termitidae*, is believed to have developed from a "rhinotermitid-like" form [Ahmad(1)].

The classification used here follows Ahmad (1), Emerson (29), and Snyder (99) and differs somewhat from that presented by Grassé (39). Both systems agree as regards *Mastotermitidae*. *Kalotermitidae* Enderlein (99) is comparable to *Calotermitidae* Sjöstedt (39). On the basis of the imago-worker mandibles, Ahmad (1) concludes that *Hodotermitidae* Snyder includes two distinct groups which apparently arose from a common stock. Grassé assigns these to different families: *Termopsidae* Grassé, including the wood-dwelling species, and *Hodotermitidae* Holmgren, including the harvesters.

In *Rhinotermitidae*, Grassé includes the subfamily *Serritermitinae* which American workers consider a subfamily of the *Termitidae*. The genus *Pro-rhinotermes* is placed in *Coptotermitinae* by Grassé and in *Rhinotermitinae*

¹ The survey of the literature pertaining to this review was concluded in December, 1958.

by Snyder (99). The subfamily Arrhinotermitinae is not included in the later classification, the species being assigned for the most part to *Prorhinotermes*.

In Termitidae, Grassé assigns *Microcerotermes* to a separate subfamily, Microcerotermitinae Holmgren. American workers, including Hare (52), place this genus in Amitermitinae. Microcapritermitinae Holmgren of Grassé (39) is roughly comparable to Termitinae Sjöstedt. Grassé & Noirot (47) have subdivided this group, placing a number of the more primitive genera (all Ethiopian) in the Apicotermitinae and assigning the balance to Termitinae. Macrotermitinae is comparable in the two systems.

Nasutitermitinae is also comparable in the two systems with the exception of the soldierless genera *Anoplotermes* and *Speculitermes*. These are assigned to the Amitermitinae by the American workers. Goetsch (36) described a nasute soldier from laboratory groups identified as *Anoplotermes*, but some workers have concluded that he must have been mistaken in his material (1, 77). Holmgren (62, 63) was very positive about the relationships of these genera to *Subulitermes*. The imago-worker mandibles of *Anoplotermes* and *Speculitermes* figured by Ahmad (1) are certainly similar to those of *Subulitermes*, but they are more similar to those of *Eurytermes* and *Euhamitermes* (Amitermitinae), to which he relates them. On the basis of the soldier mandibles, Sands (96) modifies Ahmad's scheme for Nasutitermitinae. He concludes that there are more instances of parallel evolution among the advanced genera than are indicated by Ahmad. These parallel lines, based upon morphology, correspond to the geographical distributions of the various genera.

Noirot (91) reports a reduction by one in the number of antennal segments at the molt giving rise to the nasute-nymphs of *Nasutitermes arboreus* (Smeathman), *Nasutitermes maculiventris* (Sjöstedt), and *Coarctotermes tenebricus* (Silvestri). This reduction results from a fusion of segments three and four of the prenasute-nymph. Such a reduction is not usual: the antennae generally gain segments at each ecdysis [Fuller (34)]. This same reduction has been reported for the nasute-nymphs of *Constrictotermes cavifrons* (Holmgren) [Emerson (22)] and *Tenuistrotermes tenuistrotris* (Desneux) [Weesner (100)]. All of these species belong to the *Triacitermes* branch of the Nasutitermitinae. It would be interesting to determine whether a similar reduction occurs in species of the *Paracornitermes* branch, in which it is generally agreed the nasute soldier evolved independently.

DISTRIBUTIONS AND GEOGRAPHICAL ORIGINS

The following summary is based upon Emerson (29) and is primarily concerned with families, subfamilies, and cosmopolitan or tropicopolitan genera. In considering distributions and origins of the Isoptera, one should remember that although dispersion results from winged forms, flight is relatively weak, the male cohabits with the female, and pairing occurs after flight. These features necessitate distribution over continuous or nearly continuous land masses. Thus the primitive relic genera *Stolotermes* and *Poro-*

termes may have originated on, or been distributed via, a temperate, antarctic land mass in the Triassic Period. The interdistribution of Neotropical and Indomalayan or Ethiopian groups was via a tropical Bering land bridge in the early Cretaceous Period. More limited distribution of some forms may have been effected in floating logs.

The primitive Mastotermitidae is well represented in the fossil record. It was apparently cosmopolitan in the Mesozoic and Tertiary periods and may go back to the Permian. The single living species (*Mastotermes darwiniensis* Froggatt) occurs in eastern and western Australia, north of the Tropic of Capricorn, where it competes successfully with many highly evolved forms. Hodotermitidae is morphologically primitive and has many representatives in the fossil record, including modern as well as extinct genera (23, 29, 99). The Termopsinae probably arose in temperate Eurasia in the early Mesozoic Period. Of surviving genera, three are Palaearctic (temperate and subtropical) and one is Nearctic. *Stolotermes* (Stolotermitinae) is south temperate with five Australian and one Ethiopian species. *Porotermes* (Porotermitinae) is also south temperate with one species in each of the Ethiopian, Australian, and Neotropical regions. Of the Hodotermitinae, *Microhodotermes* probably originated in the subtropical Palaearctic and spread to the Ethiopian region. *Anacanthotermes* is thought to have originated in the Palaearctic steppes and to have moved to the Indomalayan region. The third living genus, *Hodotermes*, is Ethiopian. Of the Kalotermitidae (9 genera, 331 species), the most primitive forms and all but one genus occur in the Neotropical region which must represent a primary or secondary source of distribution. *Kalotermes* and *Neotermes* are cosmopolitan, and *Glyptotermes* is tropicopolitan. These three genera are represented in the fossil record, as is also *Cryptotermes*. In the tropics the members of this family tend to be peripheral in distribution.

Rhinotermitidae (13 genera, 166 species) probably originated in the Indomalayan region. *Coptotermes* occurs in all regions except the Nearctic. *Heterotermes* is thought to have originated in the tropics in the late Jurassic or early Cretaceous Period and occurs in all regions except the Palaearctic and Malagasy. *Reticulitermes* was a temperate genus in the Tertiary and is now restricted to the Palaearctic and Nearctic regions. The most primitive Rhinotermitinae are Indomalayan and the most highly evolved are Neotropical.

Among the subfamilies of Termitidae, the Amitermitinae (21 genera, 345 species) apparently originated in the Indomalayan region. *Amitermes* occurs in all regions except the Papuan, with the most primitive species in the Indomalayan. *Microcerotermes* occurs in all regions except the Nearctic and probably originated during the Cretaceous Period in Africa. The Termitinae (56 genera, 345 species) apparently originated in Africa during the same period. The most primitive species of the tropicopolitan genus *Termes* are found in this area. The Macrotermitinae (12 genera, 277 species) are confined to the Ethiopian, Indomalayan, and Malagasy regions. They are

believed to have originated in the Ethiopian region during the Oligocene and moved to the Indomalayan region during the Miocene. The occurrence of five endemic species of the highly evolved genus *Microtermes* in Malagasy "is one of the most baffling of all the cases of anomalous distribution" [Emerson (29)]. Nasutitermitinae (48 genera, 550 species) apparently originated in the Neotropical region before the Cretaceous Period. The Indomalayan region was a secondary source of distribution. The tropicopolitan genus *Nasutitermes* apparently arose in the Neotropical region and then moved to the Indomalayan and on to Africa. In Africa this genus does not compete ecologically with *Trinervitermes* which seems to have emerged during the Miocene Period in the Ethiopian savannahs and was then dispersed to the Indomalayan region during the Miocene or Pliocene. The Seritermitinae is represented by a single Neotropical species.

It is interesting to note that the morphologically primitive forms which have survived are temperate or subtropical in distribution (Termopsinae, Stolotermitinae, Porotermitinae), or are able to survive under relatively dry situations (Kalotermitidae), or have a biology which differs from what was presumably the primitive situation (*Mastotermes* and Hodotermitinae).

NESTS AND FEEDING

Earlier studies of termite nests and feeding are extensively discussed and illustrated by Hegh (57) and summarized by Grassé (39) and Grassé & Noirot (49). Various aspects of nest structure have been reviewed by Emerson (25, 26, 31). Apparently certain behavioral patterns affecting nesting and feeding habits have evolved independently in many diverse genera (26, 49). The wood-dwelling and wood-eating habits of the Termopsinae and most of the Kalotermitidae probably represent the primitive condition (26).

In several instances morphologically primitive species have specialized and complex nesting and feeding habits. *M. darwiniensis* is basically a wood-eating, subterranean-dwelling termite which constructs above-ground passageways and has a biology similar to the Rhinotermitidae [Hill (61)]. It has been reported attacking a variety of cellulose and animal products (leather, ivory, etc.), which is also true of many rhinotermitids. Species of the Hodotermitinae are subterranean in habit and construct complex subterranean nests. In *Hodotermes* these are composed of earth, saliva, and fecal carton [Coaton (17)]. The nests are not surrounded by an air space; they are subspherical in shape and may be up to 2½ ft. in diameter. They consist of a series of thin horizontal shelves supported by numerous pillars, and the various levels are connected by short inclines [Coaton (17); Hegh (57)]. A single colony has a series of such nests with numerous subterranean runways, grass storage areas, and surface earth dumps (17). No above-ground passageways are constructed. The pigmented workers forage freely except on very hot or cold days. Surface openings are plugged when not in use, and the colonies can withstand considerable periods of flooding. Grasses are cut and stored. It is interesting to note that the foragers are not usually

accompanied by soldiers, which are largely confined to the subterranean workings [Coaton (17)].

Other Termopsidae are wood-dwelling, wood-eating forms and are apparently dependent upon a very moist situation [Castle (9); Coaton (14); Hill (61); Imms (64)]. The nest consists of excavations in the wood in which the primary pair established the colony. Fecal material may be employed in walling off certain areas. They are not known to penetrate soil or to build covered ways.

Most Kalotermitidae are wood-dwelling, wood-eating forms and are able to survive under drier situations than other termites. Many may attack living trees, which they usually enter via areas of dead wood (*Neotermes*, *Kaloterms*, *Glyptotermes*). *Neotermes tectonae* (Dammerman) is apparently unable to survive in dead trees and the colonies die out about three years after timber is girdled or felled [Kalshoven (66, 67)]. Certain *Cryptotermes* may also attack living trees, but many survive under extremely dry conditions and some tend to attack wood protected from moisture [Banks & Snyder (2); Coaton (15)]. *Paraneotermes simplicicornis* (Banks) is the only species of the Kalotermitidae known to be subterranean in habit. It nests in buried wood and attacks wood lying upon or buried in the soil. It is not known to build covered ways [Light (76)].

Members of the Rhinotermitidae are basically subterranean termites. They penetrate soil and usually construct covered passageways above the surface of the ground. The nest is commonly located in buried wood but may be above the ground in moist situations. Certain *Coptotermes* construct earthen mound nests, usually centered in a stump [Calaby & Gay (8)]. *Coptotermes formosanus* Shiraki may construct definitive wood or fecal carton nests either in the soil or on house rafters. It may also nest in ships, inhabiting the moist timbers below the water line [Oshima (93)]. *Schedorhinotermes* and *Prorhinotermes* also construct carton nests, usually within hollows in trees [Coaton (16); Grassé (39); Light (75)].

The Termitidae are basically subterranean termites. Many have arboreal nests but these are usually connected with the ground by covered ways and the entire colony may move into the soil during the dry season. It seems probable that all young pairs nest in a simple cavity in the soil (the copularium). The mature nest, whether subterranean, mound, or arboreal, is constructed by the individuals which the pairs produce. Many members of the subfamily Amitermitinae, including *Gnathamitermes* and most *Amitermes*, nest in simple excavations in the soil (2, 26, 82). Other *Amitermes* build earthen mounds which usually have a typical form for a given species (25, 26, 39, 57, 59, 61). *Amitermes excellens* Silvestri constructs earth carton nests on the sides of tree trunks. The nests have chevron-shaped extensions, reaching outward and downward, which apparently serve as rain-shedding devices [Emerson (26)]. Most species of *Amitermes* construct covered ways to reach food above the ground although *Amitermes wheeleri* (Desneux) apparently does not (2, 82). Some *Amitermes* attack solid wood, others rotten wood, and

some the weathered film of surface wood. Others feed upon vegetable debris or harvest and store grass and leaves (2, 59). *Drepanotermes* construct low earthen mounds and harvest grass, collecting food "by day as well as by night" [Hill (59)]. Certain species of *Microcerotermes* construct nests of earth, saliva, and fluid fecal material (16, 26) and others of fecal ("stercoral") material only (39, 49). The nest may be arboreal, upon the surface of the ground, or partly or completely subterranean, depending upon the species, the climate, and the time of the year (16, 26, 39, 57, 60, 102). Many species attack wood and some are reported to feed on vegetable debris (16, 54, 60).

In the subfamily Termitinae, a number of genera are humivores with entirely subterranean workings. These include those genera which Grassé & Noirot (47) assign to the Apicotermiinae, along with others too numerous to list (49). Frequently the nests are simple excavations in the soil. In *Apicotermes* the nests are very precise and complex and have been the subject of much recent investigation (19, 20, 21, 30, 47, 57, 97, 98, 102, and others). The nests are ovoid and may be up to 42 cm. long (97). They are surrounded by an air space and the outer wall is usually perforated by a series of regularly arranged pores whose structure varies with the species. The outer nest wall consists of sand grains and the inner floors are apparently fecal material. Internally the nest has a series of horizontal floors connected by straight or spiral ramps. The floor levels are visible externally. The nests of some species have circular tubes enclosed in the nest wall into which the external pores open. These pores are not large enough to permit passage of the termites and are thought to serve as a means of nest ventilation. Each colony apparently has a series of interconnected nests. The details of nest structure present a visible record of behavioral patterns which may be arranged in a phylogenetic sequence [Schmidt (97)]. Variations in nest structure have been used as a basis for distinguishing two ethospecies which are morphologically similar [Emerson (30)].

Cubitermes and *Thoracotermes* build earth carton nests above the ground and are listed as humivores by Grassé & Noirot (49). The nests of *Cubitermes* are frequently mushroom shaped, the cap apparently serving to shed the rain [Emerson (25, 26, 31); Grassé (39); Hegg (57)]. *Cubitermes finitimus* Schmitz may construct nests on the sides of tree trunks. These nests have a series of semicircular caps, one above the other, and may be associated with a number of free "mushroom" nests at the base of the tree [Emerson (31)]. Other forest-dwelling species construct similar nests (26, 31, 57). *Proculitermes* also construct nests of earth carton on tree trunks. In these, a series of hollow tubes are arranged in a chevron pattern on the trunk above the nest proper. The lateral tubes extend down from a central, perpendicular tube [Emerson (26, 31)]. The tropicopolitan genus *Termes* shows a variety of nesting patterns from low mound nests to arboreal nests. Two species have only been collected from the arboreal nests of specific *Constrictotermes* [Emerson (26)]. [*Termes* species described as fungus growers by Coaton (16) = *Odontotermes* (Macrotermiinae) (99)].

The structure of the nests of the Macrotermitinae is probably the most completely documented (16, 37, 38, 39, 43, 44, 46, 49, 53, 57, 68 to 72, 85, 87, 89, 102, and others). All species construct fungus "combs" of fecal material, and in all except *Sphaeroterme sphaerothorax* (Sjöstedt) [Grassé & Noirot (43)] fungi flourish upon these combs. The actual role of the fungi in the biology of the termites remains undetermined. They were once thought to be completely incidental but are now believed to contribute certain food elements, possibly vitamins [Grassé (38); Sands (95)]. Lüscher (85) found that termites in culture did not feed extensively upon the fungi, but once a conidium was attacked it was quickly consumed. Sands (95) found that groups of *Odontotermes* survived longer in the laboratory if provided with portions of fungus combs. Lüscher (85, 87, 89) has suggested that the fungi (or associated bacteria, or both) raise the temperature in the central portion of the nest and promote air circulation through the nest channels. Grassé & Noirot (49) do not agree with this interpretation. Both Lüscher and Grassé & Noirot found that the temperature in the vicinity of the combs was about 30° C. and was independent of external nest temperatures.

Most of the Macrotermitinae nest completely beneath the ground surface. Some *Odontotermes* and many *Macrotermes*, however, construct surface mounds. These vary in structure among species and within the same species in different areas. Harris (53) concludes that the latter variation reflects a response to local conditions of soil and climate and does not necessarily indicate a fundamental difference in behavior. The fungus-growing termites feed upon a variety of cellulose materials, including wood, grass, and leaves, and collect and store material in the nests [Grassé & Noirot (46); Kalshoven (72)]. They may attack wood buried in the soil or in contact with the ground, or they may forage freely above ground [Clément (11); Coaton (16); Emerson (25); Grassé & Noirot (46, 49)]. *Macrotermes carbonarius* (Hagen) constructs "highways" of earth carton over which the foraging termites move [Emerson (25)].

The habits of Nasutitermitinae vary from earth-nesting humivores or harvesters to arboreal-nesting wood eaters. The primitive *Procornitermes* are humivores, constructing nests which are very similar to those of *Apicotermes*, although without external pores. The nest is surrounded by an air space and a single colony has a series of interconnected nests. The tubes in the nest wall are perpendicular rather than circular. These nests are figured by Hegg (57) and discussed by Emerson (28). *Cornitermes cumulans* (Kollar) constructs a mound nest, the development of which has been described by Grassé (40). The nest is initially subterranean, as are probably those of most Termitidae, and is surrounded by an air space. An earthen mound is developed above the nest proper and the latter is shifted upward until it is entirely above ground. Dry grass and detritus are stored in the nests [Emerson (28)]. Other species are not known to store food and some are apparently wood eaters (28). Many Nasutitermitinae have simple excavated nests or small earthen mounds. In general, these genera are harvesters, collecting and

storing grass and leaves which are gathered by free foraging workers escorted by soldiers [*Syntermes* (27); *Tenuirostritermes* (2, 100); *Trinervitermes* (12, 39, 102); *Tumulitermes* (61)].

Grallatitermes, *Lacessititermes*, and many *Nasutitermes* build arboreal carton nests, either in bushes or at varying heights in trees (18, 26, 39, 53, 54, 57, 75). Species which construct arboreal nests in forested areas may build earthen mounds in the savannah [Harris (53)]. *Constrictotermes* construct nests on tree trunks. These nests are provided with slanted, gutter-like, solid ridges above and on the nests. Like the devices described for the nests of certain *Amitermes*, *Cubitermes*, and *Proculitermes*, the ridges apparently serve to deflect rain water [Emerson (26)]. The arboreal nests of the *Nasutitermitinae* are constructed of wood, saliva, and fecal carton. *Hospitalitermes* construct carton nests within hollows of trees and stumps. The workers, escorted by nasutes, forage freely for algae and lichens and may also attack wood. Only a thin film of surface wood is removed, apparently after it is moistened with saliva [Kalshoven (73)]. Foraging activities occur at night and into the early morning. The food is rolled into small pellets which are carried back to the nest and stored. There are indications that some division of labor occurs among the workers, some collecting and others carrying the food pellets (73). Such a division of labor may be more general than is supposed, especially in those termites having di- and trimorphic workers and soldiers.

FLIGHT

The evidence indicates that all termites produce alates which leave the colony of origin, pair, and establish new colonies. Many species have never been observed swarming and many records for crepuscular and nocturnal fliers are based solely upon captures at lights. The examples cited below have been selected from those presenting some details of meteorological conditions, extent of flight, etc. Other examples are cited in Grassé (39).

Flight is most commonly associated with rainfall. Emergence may occur as soon as the initial rains of the season have ceased if other conditions are favorable [*Reticulitermes hesperus* Banks (94, 101)] or during a subsequent drizzle [*Gnathamitermes perplexus* (Banks) (2, 82)] or heavy rainfall [*T. tenuirostris* (82)]. Flight may occur during the rainy season but not immediately after a rain [*N. tectonae* (65); *Zootermopsis laticeps* (Banks) (82)]; or it may occur just before the rains begin [*Coptotermes acinaciformis* (Froggatt) (8)]. In many dry-wood termites, especially *Cryptotermes*, flight is not associated with rain.

Flight may be diurnal, occurring throughout the daylight hours when other conditions are favorable [*G. perplexus* (2, 82)], or may be limited to a very short period just before sunset [*A. wheeleri* (82)]. Some species commonly emerge around midday or midafternoon [*R. hesperus* (94, 101)]. Some fly in the late afternoon and early evening [*C. acinaciformis* (8); *Pseudocanthotermes militaris* (Hagen) (46); *Hodotermes* (17)]. Many fly in the evening

and into the night [*N. tectonae* (65)], and others apparently fly only late at night [*Z. laticeps* (82)]. Diurnal flight is apparently controlled by the amount of illumination (in addition to humidity and temperature), and not by the time of day, since alates will emerge when artificial illumination occurs under laboratory conditions [Herfs (58)]. Similarly, evening fliers emerge earlier on cloudy days or if the nests are covered [Kalshoven (65)].

Flight is initially dependent upon the presence of alates which usually appear shortly before the normal emergence period. The synchronized appearance of alates at this time suggests that their production is in some way regulated by the flight pattern. Indeed, young brachypterous nymphs appear in the colonies shortly after the flight of the alates [Bathellier (3); Buchli (7); Weesner (100)]. Buchli concludes that the brachypterous nymphs are produced in response to an increased nutritional level in the colonies after the departure of the alates.

Herfs (58) analyzed alate production in large groups of *Reticulitermes lucifugus* (Rossi), maintained under constant conditions and found that the peak of alate production occurred at the same time as in field colonies, although some alates were produced throughout the year (in different colonies). In individual colonies, alates appeared within 0.25 to 3.5 months, with an average of 1.6 months for 13 groups. Buchli (7) also found that laboratory groups of *R. lucifugus* tend to have an annual cycle of alate production. Sometimes it shifted to a semiannual cycle, presumably because the development of the alates was not arrested by lower winter temperatures, as would occur in wild colonies.

Scattered flights occur in most species before or after, or before and after, the major swarm. However, the phenomenon of a general synchronized flight from many colonies subject to the same meteorological conditions has been reported for many species from all families. The most diffuse flight patterns occur when flight is not associated with rainfall. The advantages of a synchronized flight from many colonies in an area are evident. Alates are preyed upon by ants, birds, bats, and other enemies, and only a few survive to dealate, pair, and establish new colonies. Probably most of the successful matings result during massive, synchronized flights and relatively few issue from single colony or limited emergences. The extent of these flights is indicated by the following remarks: "For a distance of nearly 3 miles they were everywhere flying. . . . It is necessary to leave summer houses because of the immense hordes of these insects" [Deitz & Snyder (18)], and, "Alates occur in great numbers over many miles of country" [Calaby & Gay (8)]. I emphatically agree with Herfs (58) that it is difficult to conceive of sibling matings as "being the rule" (2, 39, 46, 91) if synchronized flights occur, even infrequently, in any area where numerous colonies occur. The erratic directions of flight would tend to mix alates when large numbers are emerging from many colonies.

Pairing behaviors have been summarized by Grassé (39), and only a few warrant discussion here. The basic pattern involves flight, dealation (which

may follow pairing), seeking on the part of the male, and "attractive" behavior, probably involving emission of a scent, on the part of the female. When the female has been joined by the male, the two then seek a nesting site, moving in tandem with the male following the female. In *N. tectonae*, however, either sex upon dealation seeks out a nesting site [Kalshoven (65)]. Having found a suitable site (usually a hole in a dead area of a living tree), they proceed to "attract" other primaries by extending the end of the abdomen through the opening. This "calling" behavior may continue over a period of weeks or even months until the first individual is joined by one of the opposite sex; the opening is then sealed. This type of pairing behavior may be correlated with the diffuse flight pattern which occurs in this species [Kalshoven (65)].

Grassé & Noirot (46) report a very interesting pairing situation in *P. militaris* in which the females were found fluttering to the ground with dealated males clinging to their abdomens. If such pairing occurs before flight, then sibling matings might well be the rule in this species. If, however, flight of either male or female or both precedes pairing and the male then dealates, clings to the female, and she again flies off, crossbreeding would still be favored. This latter pattern of pairing was described by Fuller (32, 33) and is illustrated in Hegh (57). Fuller (33) states that "in every case mating occurs after the insects come to rest."

CASTES

Caste determination has been reviewed recently by Brian (4) and the discussion here is primarily concerned with other aspects and subsequent papers. For remarks regarding alates see Brian (4) and the section on flight.

Soldiers.—Soldiers occur in all known genera except *Anoplotermes* and *Speculitermes*, in which they have apparently been lost. Soldiers are usually apterous but may have wing buds and can probably be derived from any individual which is capable of molting [Noirot (91)]. The soldier is preceded by a soldier-nymph (either mandibulate or nasute) which is apparently fixed in its direction of development. It has the general form of the soldier and a relatively constant and short stadium (5, 9, 51, 65, 71, 84, 94, 101).

In Termopsinae and Kalotermitidae, the soldiers are polymorphic, representing different instars. In general, the older the colony, the larger the soldiers. In *Zootermopsis*, soldiers of various instars and distinct sizes are produced during an annual flare of soldier production. The number of soldiers is reduced by cannibalism, and the small individuals are destroyed in favor of the larger [Castle (9)]. The more uniform soldiers of mature *Kalotermes* colonies are probably also in various instars but since the "pseudergates" [Grassé & Noirot (42)] from which they derive are relatively stable in size, the soldiers are not conspicuously different from one another. In the Rhinotermitidae, di- and trimorphic soldiers have been described for *Pseudotermes*, but these are probably polymorphic soldiers in the sense used above [Clément (10)]. The soldiers of the Heterotermitinae are poly-

morphic. In the Rhinotermitinae a true dimorphism occurs with large mandibulate and small nasutoid soldiers. In the Termitidae there are many instances of dimorphic soldiers, although they are usually of the same type (mandibulate or nasute). In some of these a sexual dimorphism occurs [Noirot (91)], in others the soldiers arise in two (or, rarely, three) different instars and are essentially polymorphic soldiers which are regularly produced and maintained by the colony.

In young colonies the soldiers are smaller than those in mature colonies. In the lower termites this results from an earlier development of the soldier (5, 9, 48, 65, 94, 101). The first individual to enter the fourth stadium [or possibly the third in *Kalotermes flavicollis* (Fabricius) (48)] may be a soldier-nymph. It has now been established that a later third-instar young may become a soldier-nymph even when older individuals are present in the colony [Buchli (5); Grassé & Noirot (48); Light & Weesner (84)]. On the other hand, the initial soldier-nymph in young colonies of *Zootermopsis nevadensis* (Hagen) may be fifth- and even sixth-instar young instead of the usual fourth (personal observations). Coaton (17) attributes the small size of the initial soldiers (and workers) of *Hodotermes* to a limited nutrition. As in Termitidae, the colonizing pair apparently does not feed until the first brood is reared.

Usually only one soldier is produced in the young colony in lower termites. Upon removal of the initial soldier, Grassé & Noirot (48) obtained a replacement soldier in each of five colonies of *K. flavicollis*. Thus, as has been demonstrated in *Zootermopsis* [Castle (9)] and in *R. hesperus* [Light & Weesner (84)], more than one individual is capable of becoming a soldier. The presence of one soldier in some way inhibits the production of additional soldiers in the young colony. Although soldiers were not produced in single-young colonies of *R. hesperus* [Weesner (101)], one was obtained in *K. flavicollis* by Grassé & Noirot (48). I have recently obtained soldiers in a number of single-young colonies of *Z. nevadensis*. These differences may indicate a modification of the mechanism of soldier production between *Reticulitermes* and the more primitive genera or they may reflect the lower incidence of soldier production in young colonies of *R. hesperus*.

It is important to note the accumulating data regarding the incidence of a soldier among first-year colonies. The earlier concept that all normal colonies produce a soldier from the first young [Light (78)] has not been supported by recent work [Buchli (5); Grassé & Noirot (48); Light & Weesner (84); Weesner (101)]. The earlier concept arose from work with *Zootermopsis*, which has a high rate of initial soldier production. Apparently the tendency to produce a soldier from the initial group is much lower in *Reticulitermes* (5, 84, 101). Grassé & Noirot obtained soldiers in 65 per cent of their young colonies of *K. flavicollis*. If a soldier is not produced from the first young of all colonies, soldier production must be controlled by some positive environmental effect as opposed to the negative effect of inhibition which is exerted once a soldier is present [Grassé & Noirot (48); Noirot (91); Weesner

(101)]. There is also evidence that soldier production may vary with the source colony of the alates [Light & Weesner (84)]. Furthermore, colonies which have produced one soldier show a higher tendency to produce a replacement soldier when compared with the production of the first soldier in the same series of colonies [Grassé & Noirot (48); Light & Weesner (84)]. These conclusions are based upon small numbers and need further investigation.

It seems unlikely that the initial soldier represents a special case of soldier production, as suggested by Grassé & Noirot (48). It is true that the soldier-to-nonsoldier ratio is very high in young colonies having a soldier, but this ratio is apparently controlled by a threshold effect after a soldier is present. Thus soldiers are produced in mature colonies in the presence of other soldiers, presumably when the soldier-to-nonsoldier ratio has fallen below a certain level.

Lüscher (90) has obtained interesting results with brachypterous nymphs of *K. flavicollis* injected with active corpora allata of functional neotenic. At the subsequent molt one was undifferentiated, one became a neotenic, one a soldier-neotenic, and the last eight to molt became soldier-nymphs. No such incidence of soldier production occurred in the controls. Thus soldier production may be correlated in some way with an activation of the corpora allata during a certain period of development.

In the Termitidae the dwarfed young of small colonies are apparently in the same stadium as their larger counterparts in mature colonies [Emerson (24); Light & Weesner (83); Noirot (91)]. Their smaller size is attributed to a limited nutritional supply. In some species several mandibulate soldiers are produced from the first group of young [Noirot (91)]. In *T. tenuirostris*, from 20 to 40 nasutes are produced from the initial group of about 70 young [Light & Weesner (83)].

Workers.—Functional workers occur in all termite colonies. When considered as a caste [Castle (9)], the term "workers" can be applied only to terminal forms. Noirot (91) and others have objected to the caste designation of workers on the basis that individuals once termed workers have since been found to be capable of molting and differentiation. Perhaps it would be better to say that in these instances the term has been misapplied. The concept of the worker as a definitive caste seems valid unless it can be demonstrated that no such individuals exist. It is difficult to prove that an individual is incapable of molting, but there are many instances in which it seems rather clear that definitive workers do occur. Thus the major workers of the Macrotermitinae are apparently terminal forms, whereas the minor "workers" may molt to produce soldiers [Grassé *et al.* (50)]. The third-instar, pigmented individuals of *T. tenuirostris* and the fourth-instar, apterous individuals of *G. perplexus* and *A. wheeleri* may be validly designated as definitive workers [Light & Weesner (81, 83)]. On the other hand, certain active, pigmented individuals ("workers") of several Nasutitermitinae have long been recognized as the source of larger workers, nasutes, or both [Bathellier (3); Emerson (22)] and are, therefore, not definitive workers. In

many Termitidae the functional or definitive workers, or both, of the third or later instar display distinct morphological characters such as pigmentation, elongation of appendages, etc. [Bathellier (3); Noirot (91)]. In others, however, even the definitive workers cannot be so distinguished (as in *G. perplexus* and *A. wheeleri*). Pigmentation and an elongation of appendages are generally associated with a free foraging habit and are not necessarily indicative of a terminal individual.

In general, apterous individuals perform most of the work of the mature colony. Brachypterous nymphs, however, will care for eggs and young and will groom one another. In the lower termites, brachypterous nymphs can be maintained independently of apterous individuals. In the young colony, the primary pair excavate the original cavity and care for the eggs and young until the work is assumed by the first of the third-instar individuals.

In species of Termopsinae and Kalotermitidae, it is generally recognized that the bulk of work is performed by apterous nymphs. Grassé & Noirot (42) have termed "pseudergates" apterous individuals of *Kalotermes* of the fifth and later instar which are relatively stable in their development and molt from time to time without any appreciable change in size. They develop from apterous nymphs (larvae) or, by regression, from brachypterous nymphs (nymphs), and may become soldiers, alates, or neotenics. Grassé & Noirot have suggested that the "broad-headed" nymphs of *Zootermopsis* [Light (77, 78)] are comparable individuals. These are in the ninth or later instar but have the same origins and potentialities as the pseudergates of *Kalotermes*. Buchli (6) suggests that apterous individuals of *Reticulitermes*, which were formerly designated as workers, are also pseudergates since it has now been demonstrated that they are capable of molting and differentiation. The term "pseudergate," therefore, defines a rather large group of relatively stable individuals which function as workers but are capable of molting and differentiation. Earlier young are not included, since they cannot be derived by regression from brachypterous individuals and show a rather marked size increment at each ecdysis. Many of these younger individuals are, however, functional workers.

In the Hodotermitinae there are pigmented individuals which are apparently true workers, although no details are available regarding their origins and potentialities. "Larval workers" forage for grass along with the mature individuals, especially in young colonies [Coaton (17); Hegh (57)].

The apterous individuals of the Termitidae differ from those of the lower termites in their dichotomy from the alate line at the first molt [Bathellier (3); Holmgren (63); Light & Weesner (81, 83); Noirot (91)]. The pattern of development is usually relatively rigid, and the number of molts limited as compared with the indefinite number in the lower termites. In many species "dimorphic" workers have been described. These may represent successive stadia, and if so, only the terminal individual is a true worker. Some, however, exhibit sexual dimorphism which would permit more than one terminal worker type [Noirot (91)].

Replacement reproductives.—Most of the recent literature on replacement

reproductives has been reviewed by Brian (4), and the following discussion is intended as a supplement to this earlier paper. Instances of neotenics encountered in the field are too numerous to cite, but a rather complete list may be obtained from Snyder (99).

In the lower termites, neotenic reproductives are readily produced from apterous or brachypterous nymphs when one or both of the functional reproductives are lost from the colony. In *Zootermopsis* numerous neotenics are produced and retained, and there seems to be a higher tendency for apterous rather than brachypterous individuals to become neotenics [Castle (9); Light & Illg (80)]. "Soldier neotenics" are encountered quite commonly in field colonies of *Zootermopsis* [Heath (56)] and may be readily produced if isolation occurs during the annual flare of soldier production (personal observation). "Soldier neotenics" develop from presoldier-nymphs, and the mandibles and head are soldierlike although not as elongated as in the soldier-nymph (56). In *Kaloterme*s numerous neotenics may be produced but their number is reduced to a single pair, apparently by cannibalism [Grassé & Noirot (41); Lüscher (86)]. Kalshoven's data for *N. tectonae* (65) and Light's data for *P. simplicicornis* (76) suggest a similar situation in these species. Lüscher (88) obtained inhibition of neotenic reproductives in *K. flavicollis* by feeding the head and thorax of functional neotenics to receptive individuals. Male substance alone did not inhibit neotony; female substance alone produced some inhibition, and both male and female substance together produced complete inhibition. Lüscher's data are much clearer than the more limited inhibition that Light (79) obtained by feeding water extracts of head and thorax in *Zootermopsis*.

In Rhinotermitidae the production of "pairs" of neotenics has been described for isolated laboratory groups of *R. lucifugus* by Ghidini (35), although wild colonies are known to support a number of neotenics. Pickens (94) noted the destruction of supernumerary reproductives (primaries in this case) in coalescing colonies of *R. hesperus*. Buchli (6) has obtained neotenics in young colonies of *R. lucifugus* which still possessed the primary pair when the "workers" were "perturbed by manipulation." In these colonies the primaries were subsequently lost, apparently through neglect, in favor of the neotenics. Many authors have remarked on the infrequent incidence of primary reproductives in wild colonies of *Reticulitermes*. Their subterranean and relatively diffuse workings, however, make it difficult to find such forms. Furthermore, their workings favor accidental fragmentation of the colonies with subsequent development of neotenics from among the individuals of the orphaned groups. Actual migration and fragmentation of colonies (sociotomy) such as Grassé & Noirot (45) observed in *Anoplotermes* and *Trinervitermes* cannot be ruled out, but accidental fragmentation seems more probable in this genus. Certainly the occurrence of primary-headed colonies of *Reticulitermes* has been well documented by Snyder (2).

In the Termitidae, the neotenic forms are usually derived from brachypterous nymphs, as would be expected in view of the early dichotomy of

alate and soldier-worker lines. In some instances, however, apterous neotenic are produced after a double molt from certain apterous-line young which have relatively well-developed gonads [Noirot (91, 92)]. In the Macrotermitinae, replacement reproductives are derived from retained alates. The production of such individuals has been described in experimentally orphaned colonies in the field. [Coaton (13)] Haviland (55)]. Noirot (91, 92) states that this is the only type of replacement reproductive known in the subfamily.

Sexual dimorphism.—Foremost among the growing data indicating considerable variation in caste structure in the various groups of termites is Noirot's discovery of sexual dimorphism in the Termitidae (91). Among the species of Amitermitinae which were investigated there was no sexual dimorphism except in *Microcerotermes*. In this genus, the small "workers" are male and the large "workers" are female. Both small and large "workers" are polymorphic, representing a series of instars. Only the terminal form in either line is a definitive worker. The soldiers are usually female and are derived from third-instar functional workers. [Both Grassé (39) and Noirot (91) assign *Microcerotermes* to a separate subfamily.] Within the Termitinae a number of patterns were found. In *Termes* the "workers" are bisexual and polymorphic (third, fourth and fifth instars) and the soldiers are usually female but may be males and are derived from functional workers. In a second group (*Euchilotermes*, *Pericapritermes*, *Cubitermes*, *Noditermes*) the workers are bisexual and monomorphic (third instar) and soldiers develop from female "workers." In *Orthotermes*, however, the soldiers develop from male "workers." Finally in *Thoracotermes* there are two definitive workers, one male (third instar) and one female (fourth instar). The latter and the soldiers are derived from small female functional workers.

In Macrotermitinae there are three nonfunctional apterous instars (larvae), whereas in all others discussed by Noirot (91) there are only two. In those species having two or three categories of soldiers, the small soldier-nymphs develop directly from the larvae and the large soldiers from functional workers. In all but one species considered, the soldiers are female, the large definitive workers male, and the small definitive workers female. In *S. sphaerothorax*, however, the soldiers and larger workers are male and the small workers female. In the Nasutitermitinae which were examined the small workers are male and give rise to nasutes. The larger workers are female and in some species are polymorphic. The nasute soldiers may arise without a functional worker stage as is true of the small nasutes of *Trinervitermes* (91) and all of the nasutes of *T. tenuirostris* (100).

In the case of *T. tenuirostris* I was unable to distinguish the sexes of the nasutes or workers or to find any vestiges of gonads. Data for the proportions of nasutes to workers did not approach the 1:1 ratio which might be expected if the castes are determined along sexual lines. The proportion of nasutes to workers is very high, however, when compared with the proportion of mandibulate soldiers to workers in other species. Any differential

mortality rate, such as might occur with the increased number of molts in the nasute line (four as compared with two in the worker line), might account for a marked reduction of the expected ratio. As Noirot (91) has pointed out, it is difficult to obtain a reliable proportional sample of individuals from subterranean workings. In the largest single sample of *T. tenuirostris* there were 398 nasutes to 2109 workers. In the case of young individuals from the same colony there were 417 nasute-line young and 688 worker-line young (100). In incipient colonies the ratio of nasutes to workers was approximately 1:2 (84).

CONCLUSION

It is evident that the termites exhibit a much greater diversity of behavior, physiology, and caste structure than was realized a decade ago. Many of the apparent discrepancies in the literature are attributable to the variations which occur between different genera, species, and, indeed, between individual colonies of a given species. Many termites remain to be discovered, and as collections are broadened many taxonomic and distributional problems will be resolved. Such collections should be accompanied by most detailed information regarding nest structure, behavior, feeding, associated organisms, and so on. Observations regarding flight should be extended and placed upon a more critical basis. Culture methods must be found for those species, especially in the Termitidae, which have not yet been successfully maintained in the laboratory for any period of time. The potentialities for laboratory work are almost limitless and should be correlated, whenever possible, with data from wild colonies.

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BIOLOGY OF FRUIT FLIES^{1,2}

BY L. D. CHRISTENSON AND RICHARD H. FOOTE
*Entomology Research Division, Agricultural Research Service,
U. S. Department of Agriculture*

INTRODUCTION

The family Tephritidae (Trypetidae according to European and early American authors) is moderately large, with about 4000 species distributed throughout the temperate, subtropical, and tropical areas of the world. Individual flies vary in body length from 1 to over 20 mm. The wings of most species are pictured with yellow, brown, or black stripes or spots, or a combination of both, in characteristic positions or with light or hyaline spots in a darker field. The females of most species insert their eggs in living, healthy plant tissue. The larvae live and feed in the stalks, leaves, fruit, flower heads, or seeds. Some form galls and others are leaf miners. The fruit flies yearly take a heavy toll of fruits and vegetables in many regions of the world. Barriers to trade in fresh-food commodities, difficult quarantine and regulatory problems, prevention of development of desirable crop, and costly survey, control, and eradication programs are undesirable by-products of the depredations of these pests.

The species of the family Tephritidae fall into two broad morphological categories, one of which contains *Dacus* and its relatives (including *Toxotrypana*), the other comprising all other genera. Almost without exception, larvae of members of the former group live in, and often seriously damage, all kinds of fleshy tropical and subtropical fruits and some vegetables. The second group contains an extremely wide range of morphological characters and biological habits. It may be subdivided into two important parts—one of these is essentially the subfamily Tephritinae, which is distinctive morphologically and composed of species almost all of which habitually oviposit in the flower heads of composites and other plants. The larvae of these flies attack the ovaries and seeds of their host plants; little is known about the extent of their economic importance. The species of the second category outside the Tephritinae present an extremely wide range of habit and appearance and have been grouped in various ways by taxonomists. Their attempts to

¹ The survey of the literature pertaining to this review was completed in June, 1959.

² Unpublished biological data developed by K. L. Maehler, N. E. Flitters, C. J. Davis, L. F. Steiner, Susumu Nakagawa, Shizuko Maeda Mitchell, and their associates at the United States Department of Agriculture Fruit Fly Laboratory, Honolulu, Hawaii, are gratefully acknowledged. A comprehensive review of the world-wide literature on fruit-fly biology has not been possible because of limitations on time and space. For the most part, the examples selected to illustrate various aspects of fruit-fly biology have been species or groups which have been of special interest or concern to the authors.

correlate biological characters with genera in the main have not been successful. The last-named group contains several genera of considerable economic importance because of the habit of their larvae which, like *Dacus* species, live in crops of fleshy fruit. Among these, the genera *Ceratitis*, *Anastrepha*, *Rhagoletis*, *Euleia*, *Epochra*, and several others, are of most concern to commercial fruit growers in various parts of the world.

LIFE HISTORY

In the typical developmental cycle, adult fruit-fly females insert their eggs beneath the skin of suitable hosts, especially in ripening or ripe fruits and vegetables. The larvae shed their skins twice as they feed and grow. At completion of the third instar, the larval skin hardens to form a puparium with an inactive fourth-instar larva inside. Eventually the larva within the puparium sheds its skin, forming a pupa; pupation usually takes place in the soil. After the few days to a week or more required for attainment of sexual maturity after the adult emerges, mating occurs, and a new cycle is begun.

Adult.—Emerging adults tend to crawl upward through the soil although not always vertically. They also appear to take advantage of any cracks or crevices that might afford egress to the surface, especially when the soil is hard and compacted. Adults of *Anastrepha ludens* (Loew) have been known to emerge from fruit buried more than 18 in. below the ground surface (40). However, under normal conditions most species are seldom required to emerge from depths greater than 1 to 2 in.

A marked diurnal periodicity in emergence of several fruit flies has been noted. Approximately 95 per cent of the emergences by *A. ludens* during a two-month period occurred between 6 and 10 A.M. (40). Under laboratory conditions this rhythm appeared to be correlated with exposure to sunlight and rising temperature. A similar periodicity has been observed in laboratory studies of *Dacus dorsalis* Hendel, most of the flies emerging between 8 and 10 A.M. (61a). Overcast, rain, or low temperature sometimes extended the period but seldom beyond midafternoon. Lathrop & Nickels (35) found that *Rhagoletis pomonella* (Walsh) emerge most heavily between 7 and 10 A.M. Depending upon the type of soil, adults required from 14 to 70 or more minutes to reach the surface after bursting the puparium. It is interesting to note that most of the propellent action through the soil was by contraction and elongation of the body and ptilinum rather than by movement of the legs.

Attainment of sexual maturity and a high level of reproductive efficiency in several of the fruit flies investigated depend upon ingestion by adults of foods containing essential nutrients during a postemergence period. Certain protein hydrolysates are now known to contain the nutritive elements required by some species [Hagen & Finney (26); Hagen (24); also (61a)]. The avidity with which fruit flies consume these protein materials and their attractiveness in olfactometer experiments conducted by Gow (23) or when combined with phosphatic insecticides in bait sprays [Steiner (57, 58)] suggest that these materials or specific nutrients in them, which the fruit flies may need, strongly influence adult choice of food.

Various foods may be eaten by adult fruit flies, including glandular secretions of plants, nectar, and plant sap exuding from trunk, stem, leaf, or fruit injuries, such as those caused by the feeding of insects, by diseases, or by mechanical damage. Rotting fruits, bird dung, and decaying insects are other food sources. Honeydew secreted by homopterous insects is considered to be an especially important food as a result of Hagen's discovery that the secretions of a scale insect provide hydrolyzed proteins, minerals, and certain B-group vitamins required by *D. dorsalis* for normal fertility and fecundity, while honeydew from another homopterous insect stimulates egg production by *A. ludens* (24, also 61a). Subsequent studies at the United States Department of Agriculture fruit-fly laboratory in Honolulu have shown that *Coccus viridis* (Green) honeydew may also provide a sufficient diet for *D. dorsalis*. Honeydew is eaten avidly by most of the fruit flies studied extensively in the field, but Skwarra [see Baker *et al.* (7)] considered aphid honeydew to be more repellent to *A. ludens* than otherwise. Secretions of extrafloral glands of several plants in Hawaii are commonly eaten by *D. dorsalis* and *Dacus cucurbitae* Coquillett but apparently not by *Ceratitis capitata* (Wiedemann) [Nishida (43)].

Fruit flies often explore surfaces of fruits, leaves, and other objects with their probosces and seem to be getting food, even when there appears to be nothing to feed upon. Numerous yeast cells were found by Darby & Kapp (19) in the crops upon which *A. ludens* had apparently fed in this manner. In Hawaii, caged *D. dorsalis* flies were noted by I. Keiser to eat hyphal strands of fungus growing on bread or liquids or other materials associated with this growth. In Coimbatore, *Dacus brevistylus* Bezzi was observed by Cherian & Sundaram (16) to survey the surface of the host fruit (*Coccinia indica* Wight & Arnott) minutely and then make an insertion in the skin with its ovipositor. Later the female flies returned to the puncture sites to feed on the white exudate from these punctures.

Experiments with *Rhagoletis completa* Cresson have shown that under certain conditions solid particles may be ingested, but they are always sucked up suspended in liquid regurgitate [Brooks (15)]. A limit of 2.5 to 3 mm. on the size of insoluble solid particles that may be ingested by *C. capitata* was reported by Hanna (28).

The extensiveness in nature of sources of protein-hydrolysate fruit-fly nutrients remains to be determined except for honeydew, but of some interest has been the finding in recent Hawaiian studies that *D. dorsalis* fed on bird dung will become sexually mature and lay eggs. Rotting guavas or mangoes did not appear to provide an adequate diet in limited tests. The identity of essential nutrients and the history of their occurrence in honeydew or other food sources—whether they are basic constituents or derived from subsequent microbiological or other action—present challenging objectives for further research.

Adult fruit flies require water every few days for survival, and frequently they have been observed imbibing dew or rain droplets. Undoubtedly choice of liquid foods is often dominated by the need for water rather than presence

of essential proteins. Fruit flies may feed at any time during the day, but field observations on the behavior of *D. dorsalis* have suggested that the early morning hours, immediately after a prolonged period of inactivity during the night, are largely devoted to food getting. When seeking foods, fruit flies may explore all types of vegetation, including low cover plants and shrubs, and they may not confine their searching to areas in which host plants occur.

Diet may have a marked effect on the longevity of fruit flies. In Hawaii, at a mean temperature of 80°F. flies without food or water succumbed within three days [Hagen (24, 25)]. When provided water they lived about twice as long. Addition of carbohydrate increased longevity to about 20 days for *D. dorsalis*, 35 days for *D. cucurbitae* males, 65 days for *D. cucurbitae* females, and 55 days for *C. capitata*. Addition of minerals and B-group vitamins to a diet of hydrolyzed protein, sugar, and water each, in turn, further improved longevity. In Hagen's studies the longevity of *D. dorsalis* appeared to be inversely proportional to the degree the proteins were hydrolyzed, but a well-balanced diet with protein in the form of free amino acids was not detrimental to longevity. Similar effects of diet on longevity have since been noted at United States Department of Agriculture Honolulu and Mexico City fruit-fly laboratories.

In Hawaii and Texas, in cabinets simulating various selected climates, temperature patterns with maxima above 105°F. greatly reduced longevity of *A. ludens*, *C. capitata*, *D. cucurbitae*, and *D. dorsalis* [Flitters & Messenger (22); Messenger & Flitters (41, 42)]. The extension of the life span of *D. dorsalis*, *D. cucurbitae*, and *C. capitata* held in cages at high elevations on mountains in the Hawaiian Islands, (61a) to as long as 462 days in the case of a single melon-fly female, appeared to be the result of cool temperatures that permitted ingestion of food and water at least every few days but otherwise suppressed activity. It is not anticipated that similar survival would ever occur in nature.

An interesting competition among males of *R. completa* during the mating season, with one male fighting other males away from a single walnut while waiting for a female to arrive, has been observed by Boyce (14). When a female alights to oviposit, the male becomes excited, but stands nearby until oviposition is completed. The female is then receptive and the male inserts the aedeagus after guiding the female ovipositor to a position where it can be held by the male claspers. Mating of *R. completa* requires from 2 to 15 minutes. Similar mating habits have been observed in *Rhagoletis suavis* (Loew) but Brooks (15) indicates that the female will accept copulation long before ovipositing has been completed, and a single female may alternate egg laying with periods of copulation with as many as five males before flying away.

Mating of *D. dorsalis*, which occurs at dusk, is stimulated by decreasing light intensity [Roan, Flitters & Davis (51)]. As twilight approaches, caged flies become excited and produce a high-pitched buzzing sound. This is followed by copulation, which may last from 2 to 12 hr. under laboratory conditions. Copulation has not been observed at temperatures below 60°F. Copu-

latory activity was not inhibited completely by continuous darkness or continuous light. This suggested that some reproduction may take place in the absence of the normal stimulus of decreasing light intensity in areas where evenings are too cool for mating at the usual time.

D. dorsalis mates frequently, the female at approximately four- to five-day intervals in one extensive study conducted under summer conditions in Hawaii. When provided with sexually mature females, the males will mate more often, one individual being observed to copulate almost every evening for about seven weeks. A single mating insures production of fertile eggs for life, but more frequent mating appears to be required to sustain maximum fertility.

Exclusive access to virgin females for 10 days (usually two or three matings) by irradiated sterile males prevented subsequent fertilization of the females by normal males [Steiner & Christenson (59)]. The physiological basis for the failure of ensuing matings with normal males to cause egg fertility has not been determined, but complete admixture in the spermatheca of spermatozoa from irradiated and normal males apparently did not occur.

A strong, peculiar odor is given off by *C. capitata* males as they begin to court the females [Back & Pemberton (5)]. The odor failed to attract female flies. Males kept at 58° to 60°F. did not mate or emit the characteristic odor.

Egg.—In Hawaii the first matings and egg deposition by *D. dorsalis* females usually take place from 8 to 12 days after the adults emerge, but flies fed on a special diet of enzymatic yeast and soy protein hydrolysates may lay eggs on the fifth day. Preoviposition periods for other fruit flies may be longer or shorter, depending on the species, and they may be greatly extended by cool temperatures. In some fruit flies the males appear to attain sexual maturity before the females.

Oviposition in currants and gooseberries by *Epochra canadensis* (Loew) is preceded by an inspection of the surface of the fruit (52). When the female has selected a suitable site, the ovipositor is protruded and there begins a back-and-forward action designed to break through the epidermis. After entry into the fruit is accomplished, the entire ovipositor is moved in different directions to make a suitable space for insertion of the eggs just beneath the skin. The entire process in one series of observations consumed about 8 min.

Females of *Toxotrypana curvicauda* Gerstaecker, according to Knab & Yothers (33), oviposit most actively about sunset. The entire length of the long ovipositor is frequently inserted in unripe fruit. Females are sometimes caught by the juices exuding from unripe fruit. Eggs of *Euleia heraclei* (Linnaeus) are laid on the underside of celery leaves in cavities made by the ovipositor in the epidermis [Keilin & Tate (32)]. The ovipositor of *R. pomonella* is inserted in a blueberry at an angle of 45° [Patch & Woods (44)]. A single egg is deposited—a process requiring about two minutes.

Brooks (15) has observed that eggs in *R. suavis* egg masses are placed close together about 2 mm. deep in the skin of the walnut. If other than her own puncture is used, the female will often build a mound of eggs well above

the surface. Other species, e.g., *A. ludens*, freely void eggs in cages when hosts are not provided, but the extent to which this behavior occurs in nature is not known.

According to Boyce (14), *R. completa* may lay eggs at any time during the day when the temperature is between 65° and 103°F. and the relative humidity above 60 per cent. Under field conditions females usually oviposit between 5 P.M. and darkness; light intensity apparently is a definite factor, since on overcast days or on trees shaded by caging materials, oviposition may occur throughout the daylight hours. In Hawaii, *D. dorsalis* may lay eggs at any time of the day, but there is a pronounced increase in this activity in late afternoon and early evening hours just before sundown. Cherian & Sundaram (16) have described an unusual habit of oviposition by *D. brevistylus*: the female flies off after pricking the fruit and returns later to feed on the resulting exudate before depositing eggs in the same puncture. A maximum of eight eggs has been observed in a puncture, but three or four are more common. Bristles at the end of the ovipositors of fruit flies, as noted by Severin *et al.* (54) for *D. cucurbitae*, apparently serve as tactile organs to detect suitable places to lay eggs.

D. dorsalis females make determined efforts to displace other flies of the same or different species attempting to encroach upon ovipositional sites they have selected. Several females may successively use the same ovipuncture or ovipunctures made by other species, with the result that large numbers of eggs sometimes occur in a single cavity. Allman (1) has observed that females of *Dacus tryoni* (Froggatt) lay eggs in punctures already present in host fruits much more frequently than do *C. capitata*. In addition to old stings, *D. dorsalis* oviposition sites may include depressions or cracks in the epidermis and wounds, as well as normal skin surfaces. Penetration of the ovipositor through extremely hard skins such as those of passion fruit and avocado can be accomplished, but a prolonged effort, often by several females in succession, as observed by Steiner (61a), is required. Eggs may be inserted to a depth of one quarter inch in soft fruits, such as mango, or immediately beneath the skin in others. The possibility that feeding may be required by *C. capitata* females between each oviposition is suggested by the observations of Back & Pemberton (5). Even in heavily infested trees during years of peak damage, extensive counts by Boyce (14) revealed only one to three ovipunctures by *R. completa* per walnut. This finding suggests that this species may not intentionally oviposit in fruit in which an egg mass is already present.

A detailed account of egg development in *R. completa* is given by Boyce (14). Each ovary consists of about 24 egg tubes bound by connective tissue and tracheae. Egg development begins at the apex of each tube. As the egg grows, it travels backward toward the opening of the ovary into the vagina, probably being pushed along in part by new eggs developing behind it. Dissections of gravid females of this and other fruit flies usually show eggs in all stages of development.

When development is nearly complete, the egg enters the vagina, where

it is fertilized by sperm that has been held in the spermathecae. After lubrication by secretions from the collateral glands that empty into the vagina, the egg enters the external ovipositor, which is essentially a tube, the basal part of which is membranous and the apical part rather heavily sclerotized and sharply pointed. The distal sclerotized portion may be retracted into the basal membranous portion inside the ovipositor sheath during flight and non-egg-laying periods. After a suitable site has been prepared, the eggs are forced through the inserted ovipositor and arranged in the substrate according to the habits of the species.

Observations of Boyce (14) on single eggs of *R. completa* indicate that the hatching process requires only about 1 min. The larva scratches the interior of the egg shell with vigorous movements of its mouth hooks and pharyngeal skeleton. After partially emerging through the resulting rupture, it obtains a hold on a firm portion of substrate and pulls the rest of its body out of the shell.

Immediately after hatching, fruit-fly larvae begin to feed and burrow into the pulp of the host. In some hosts the seeds may also be attacked, as, for example, when *A. ludens* infests yellow chapote.

Early laboratory studies with *E. canadensis* suggested an effect of host on duration of the egg stage; from four to seven days were required in five hosts tested [Severin (52)].

Larva.—One group of Tephritidae larvae has a shortened, barrel-shaped body with retracted head. In another group the larvae are more elongated, the body gradually tapering from a bluntly broad posterior end to a narrow head that carries a pair of mouth hooks. Almost all species causing the greatest economic damage belong to the second group. Microscopic spines are present on the dorsum of at least some of the segments, and ventrally every segment carries one to many transverse rows of coarser spines, presumably to aid locomotion. Muscles expand the pharynx, the pump by means of which liquid and semiliquid foods are ingested.

When confronted with unfavorable microclimatic conditions, the larvae of many species are able to travel considerable distances by means of a jumping action, accomplished by arching the body and catching the mouth hooks in a slight depression at its posterior end. The muscles are then tensed to such a degree that the hooks suddenly lose their hold, with the result that the larva may be catapulted for several inches. The jumping, which may be as high as 8 or 9 in. appears to be in random directions, and some larvae have been observed to travel in successive jumps for 15 ft. across concrete floors. The jumping habit is associated only with third-instar larvae, and it appears to be lacking in most, if not all, *Anastrepha* species.

Larvae of *D. cucurbitae* have been observed (3, 54) living in liquefied pulp, hanging head down with their posterior spiracles at the liquid surface. They may be startled into disappearing into the liquid, but eventually they resume this position. Some fruits are not suitable for fruit-fly development, because they are so juicy that the larvae are drowned.

Baker *et al.* (7) quote results of studies which indicate that the pH of

sour limes (about 2.0 to 2.2) is much too low to permit development of *A. ludens* larvae. There appears to be no direct relationship between the pH of various fruits and the time required for larval growth. However, pH values in the region of 3.6 seemed to be most favorable in grapefruit or sour orange, both common hosts of this fly. Darby and Kapp (19) believed pH of soils might be a critical factor in the distribution of *A. ludens*, but Baker *et al.* (7) concluded that any abnormalities resulting from pupation in acid soils are probably not great enough to restrict this species to more favorable soils.

The larval stage of *D. cucurbitae* was appreciably prolonged when the toughened skins of certain hosts, such as partially dried pumpkins, prevented ready exit of the larvae [Back & Pemberton (3)]. A similar extension of the larval stage of *C. capitata*, observed by Steiner during the 1956 to 1957 Florida eradication campaign, was a complication that had to be overcome through adjustments in treatment schedules (61a).

The host fruit may have a pronounced influence on the length of larval development in some species. In experiments conducted by Baker *et al.* (7) at 77°F. with *A. ludens* in figs, the minimum time from egg deposition to appearance of the first adult was 32 days; in mandarin and apple it was, at the same temperature, 52.8 days.

In Hawaii, development of *C. capitata* larvae was most rapid in fortified carrot rearing medium and papaya (about six or seven days at 73° to 92°F.). Longer periods were required when the food was avocado, guava, okra, or pineapple. In tests in Israel [Rivnay (50)] larval development was slowest in apples and progressively faster in pear, peach, and figs. The variety of apples also affected the larval period of *R. pomonella* [Porter (47)], but the rapidity with which the flesh of the apples disintegrated may have been a factor.

At 78.2°F., Back & Pemberton (3) recorded a developmental period of only 4 days for *D. cucurbitae* larvae in papaya, 10 to 13 days in Bartlett pears, and 7 to 17 days in cantaloupe. Severin *et al.* (54) suggested that *D. capitata* larvae mature more rapidly in fruits that decay rapidly because the liquified pulp may be absorbed through the body wall.

Pupa.—The larvae of *E. canadensis* sometimes mine beneath the skin of a gooseberry or currant, leaving a visible trail, and they commonly devour seeds as well as the pulp. When mature they cut their way to the surface of gooseberries through healthy tissue, but may also use exits made for other purposes. During a three-week observation period (40) more than 92 per cent of *A. ludens* larvae were seen to leave fruits to pupate before 9 A.M. Low temperatures, whether in the laboratory or the result of driving rainfall, stimulated their emergence. After leaving the fruit, mature larvae of *A. ludens* wandered about in a haphazard way, traveling at the rate of about 10 to 12 cm. per minute. Sometimes they pupated under leaves without burrowing, in mulch when it was present, or on the soil surface directly under fallen fruit when the ground was moist. Pupae were rarely found more than $\frac{1}{2}$ in. below the soil surface (19).

Larvae of *R. suavis* (15) remain in walnut husks until the onset of severe

freezing weather and then leave during intervening warm periods. Boyce (14) observed that larvae of *R. completa* tend to congregate in disintegrated husk tissues just before they are ready to leave. When there is only one exit hole, several days may elapse before all larvae leave the husk. Pupation occasionally occurs inside the husk, especially when the larvae are trapped there. The greatest number of *R. completa* larvae leave the walnuts between 5:30 and 8 A.M., when air temperatures range from 40° to 71°F. Third-instar larvae of *R. completa* exhibit marked geotropism, disappearing into the soil quickly after leaving the husks. Under field conditions, pupation usually occurs within 24 hr. after entry of the mature larvae into the soil [Boyce (14)] Back & Pemberton (3, 4) noted that *D. cucurbitae* takes about 1 hr. to form a puparium. At a mean temperature of 72°F. (range 66° to 78°F.), pupae within the puparia were observed at the end of 48 hr.

Baker *et al.* (7) found that there is a direct relationship between loss of moisture, e.g., body weight, and percentage of emergence of adult *A. ludens*. Furthermore, they observed that flooding of puparia may also reduce survival. McPhail & Bliss (40) concluded that moisture has little effect on the length of the pupal stage but a pronounced effect on survival.

In areas of California where walnuts are grown, most *R. completa* larvae pupate in the soil at a depth of $\frac{1}{2}$ to 2 in. [Boyce (14)]. When the larvae are not able to burrow in their normal manner, pupation occurs on the soil surface. The character of the soil and its moisture content also influence the depth at which pupation occurs. The variation in duration of stages and host preference of representative fruit flies is illustrated in Table I.

DIAPAUSE

Certain fruit flies, especially those living under tropical and subtropical conditions, have multivoltine life cycles and are not known to undergo diapause. Many other species are strictly univoltine; they appear to be restricted to portions of the world that have pronounced seasonal fluctuations in climate, and diapause is typical of these insects. Most of the North American *Rhagoletis* spp., to employ a familiar example, are representative of this type of development.

As Boyce (13) has shown for *R. completa*, in some univoltine insects the diapause is broken after the end of the first year, but it may continue for the second, third, or even until after the end of the fourth year. The factors that bring an end to diapause in flies emerging after the first winter are apparently not operative for other portions of the population, which must undergo further treatment by the environment before other adults can emerge. This type of "obligatory" diapause [Lees (36)] in the univoltine fruit flies apparently only occurs in pupae. Dean (21) observed that during wintering, pupae of *R. pomonella* invariably attain the late phanerocephalic stage [see Snodgrass (56)] before going into diapause.

Little is known about the factors controlling diapause in fruit flies. A "length of day" factor hardly seems applicable to the pupal stage, most of

TABLE I
BIOLOGY OF REPRESENTATIVE FRUIT FLIES*

Species and distribution	Hosts	Duration of stages (days)				Adult longevity	Number of eggs
		Preoviposition period	Egg stage	Larval stage	Pupal stage		
<i>Anastrepha fraterculus</i> (Wied.) (South American fruit fly). Continental America from Mexico to Argentina; also Trinidad and Tobago.	A variety of fruits and vegetables including citrus spp., coffee, <i>Prunus</i> spp., <i>Eugenia</i> spp., <i>Spondias</i> spp., grape, and guava.	—	3 days in summer; 6 in winter.	15 to 20 days in summer; 20 to 25 in winter.	15 to 18 days in summer; 20 to 25 in winter. In exceptional cases adults have been known to emerge from pupae after 12, 14, and 18 mos.	8 mos. in cages; Mexican strain, 9 mos.	Eggs laid singly; as many as 50 in single fruit.
<i>Anastrepha ludens</i> (Loew) (Mexican fruit fly). Mexico, Central America. Movement of flies into lower Rio Grande Valley of Texas, U.S.A., each winter may cause devastation in citrus fruits.	<i>Azadirachta indica</i> , <i>Linn.</i> , <i>citrus</i> , <i>Annona</i> spp., <i>Casimiroa edulis</i> Liave & Lex. (white sapote), rose apple, mango, peach, <i>Sargassum grisea</i> S. Wats (Clapote), and others.	Oviposition 1 to 8 days after mating; mating occurs 8 to 34 days after emergence. Males reach maturity before females. (7).	6 to 12 days, average about 8, natural summer temperature, Cuernavaca, Mexico (40) 3.5 to 4.5 days at 75° to 80°F. (42).	15 to 32 days depending on fruit host; at 59°F., shortest duration 54 days (7); 10 to 12 days at 75° to 80°F. Minimum food consumption 60 days at 70.5°F. (40).	17.6 days at 77°F. (7); 16 to 19 days at 75° to 80°F. (42); 12 days at 87.8°F.; 107 days at 53.4°F. (7).	2½ to 11 mos. (18). Males survived 16 mos. (7).	As many as 401 eggs from single female (40); average of 1400 eggs per female provided special protein hydrolyse diet; 2 females each laid more than 4000 eggs (48).
<i>Ceratitis capitata</i> (Weid.) (Mediterranean fruit fly). Africa, Mediterranean area, South America, Australia, certain Atlantic and Pacific Islands, including Hawaii and Bermuda.	Citrus fruits, Surinam cherry, peaches, mango, papaya, guava, loquat, rose apple, and others; more than 200 hosts in all.	4 to 5 days mean temperature 80°F., full egg laying capacity to 10 days (5). On diet of honey, protein hydrolyse, and linoleic acid, mating may occur 2 days and oviposition begin 3 days after emergence under field conditions about 7 to 9 days (61a).	2 to 4 days. May be prolonged in cool weather to 16 to 18 days before maturity occurs. (2, 5)	Normally 6 to 10 or 11 days; at temperatures 57° to 83°F., 26 to 48 hr. required for development; instar 24 to 48 hr. and third instar 48 to 265 hr.; total larval development from 24 to 50 days at 58° to 62°F. (5, 6). Rate of development greatly affected by host (49, 61a).	9 to 11 days at a mean temperature of about 76°F.; at 79.4°F.; most individuals at 78.3°F. about 60 days by cool temperature (5, 6, 61a).	About 50 per cent of caged flies dead after 2 mos., near optimum conditions. 10 mos. in Hawaii, Y. cool mountain locations (6, 61a).	Caged females as many as 800 eggs during lifetime (5); average 911 eggs per female on special diet (24); on mountain situation estimated to average about 300 eggs per female.
<i>Dacus cucurbitae</i> Coquillett (Melon fly). Africa (Kenya Tangaika), Mauritius, Philippines, Guam, Tinian, Saipan, Ryukyu Islands, Sarawak, Timor, and Indonesia.	Preferred hosts include cucumber, melon, squash, okra, and beans; more than 80 fruits and vegetables are attacked.	In mass rearing in Hawaii with special diet some eggs are laid on 9th day after mating (61a). Mating consistently occurs 10 days after emergence with first eggs on the 11th and 12th days, summer conditions Hawaii (3).	50 per cent hatch in 26 to 28 hr. under field conditions (3).	4.2 to 7 days at mean temperature of 79°F.; at 78.2°F., 4 to 10 days; at 78.3°F., Bartlett pears; 7 to 17 days in cantaloupe (3).	7½ to 13 days at temperatures of 71.6° to 79.4°F.; most individuals at 78.3°F. 79.4°F. (2); ecdisis delayed for 59 days by cool temperatures (3).	462 days, cool mountain location (61a); normally about 1 to 3 or 5 mos.	1000 during lifetime (3). Average 15 eggs per female per day for 30 days on special diet. Adults live by several days 2 weeks between ovipositions (3). One egg laid every 15 to 30 sec. after oviposition inserted, 1 to 40 eggs per cage (34).

* Data for *R. completa* compiled by Martin F. Barnes, University of California Citrus Experiment Station; for *R. pomonella* by Phillip E. Marucci, New Jersey Agricultural Experiment Station. The table also includes information compiled by the Insect Pest Survey and Plant Quarantine Division, United States Department of Agriculture.

Species and distribution	Hosts	Duration of stages (days)				Adult longevity	Number of eggs
		Preoviposition period	Egg stage	Larval stage	Pupal stage		
<i>Dacus dorsalis</i> Hendel (Oriental fruit fly). Southeast Asia and Pacific Islands, including Hawaii, Bonins, Marianas, Philippines, Formosa, and Ryukyu.	More than 150 species of fruits and vegetables including guava, mango, <i>Eugenia</i> spp., surinam cherry, citrus, loquat, papaya, and tomato.	8 to 12 days normally; flies on special diet enzymatic yeast and soy hydrolysates may lay eggs on 5th day (61a.)	24 hrs. summer conditions in Formosa; 20 days in winter (37).	9 to 35 days depending on season. When subjected to simulated climates; 6 to 7 days under optimum Honolulu, Hawaii conditions (61a). Lowest average temperature permitting this and other stages development ranged from 56.0° to 57.5° F. (41).	10 to 12 days, 65° to 85° F., fluctuating temperatures; 21 to 26 days 48° to 80° F.; may be extended to about 50 days at 54° F. (24, 61a); greatly reduced when maximum above normal field conditions (61a); on special soy hydrolysate diet average of 3200 eggs per female (29).	Approximately 1 yr. cool mountain locations; normally 1 to 3 mos.; strongly affected by diet. 32 days at 54° F. (24, 61a); greatly reduced when maximum above normal field conditions (61a); on special soy hydrolysate diet average of 3200 eggs per female (29).	As many as 3062 by a single female, 136 in a single day, eggs laid over period of 27 consecutive days; from 1260 to 1500 eggs per female (29).
<i>Dacus oleae</i> (Gmelin) (Olive fly). Mediterranean basin, Northern, Eastern and Southern Africa, Canary Islands, India, and Western Asia.	<i>Olea</i> spp.	6 to 10 days before mating in summer.	2 to 4 days in summer in Yugoslavia.	10 to 14 days in summer in Yugoslavia.	About 10 days in summer; early generations pupate mostly in fruits; last generation larvae leave fruit to pupate in soil and elsewhere.	—	10 to 12 eggs daily; about 200 to 250 in a lifetime; usually no more than 1 egg per olive fruit.
<i>Dacus tryoni</i> (Froggatt) (Queensland fruit fly). Australia.	More than 100 fruits and vegetables including papaya, citrus, guava, mango, and peach.	—	2 to 3 days laboratory conditions October, November (1).	10 to 31 days with average of 20 days under October, November, laboratory conditions.	About 7 days in summer months; more in cool weather. (61a).	Extended periods, 4 or 5 overlapping generations develop annually; passes winter (June to October) in adult stage.	As many as 67 adults may develop from a single apple (1).
<i>Eoecetra canadensis</i> (Loew) (Currant fruit fly). Southern Canada, Northern United States.	Principally currants and gooseberries	Mating 5 days after emergence, first eggs on 6th day when temperature ranged from 77° to 42° F. (52).	4 to 7 days in laboratory; 6 to 8 in field (52).	Combined egg and larval stages 19 to 32 days in gooseberry and red currant; in Maine larvae begin to emerge 1st day after mid-July to around June 19 days (52).	—	—	Females lay some eggs almost every day, as many as 33 in a single day; only one or two in each gooseberry.
<i>Euleia heracleis</i> (Linnaeus) (Celery fly). British Isles, Europe, and North Africa, recorded from Morocco, North Africa, and Asia Minor.	Mines the leaves of celery and parsnip; various species of <i>E. heracleis</i> most important wild hosts.	—	6 to 14 days.	14 to 19 days with in leaf surface.	3 to 4 weeks in summer; second brood maggots may be present following spring.	—	As many as 100 per female; usually singly on under-surface of leaves.

TABLE I (continued)

Species and distribution	Hosts	Preoviposition period	Egg stage	Larval stage	Pupal stage	Adult longevity	Number of eggs
<i>Megastelalis pardalinae</i> (Bigot) (Baluchistan melon fly). Afghanistan, India, Iran, Iraq, Jordan, Pakistan, Palestine, Saudi Arabia, Syria, Lebanon, Turkey, Transcaucasia.	<i>Citrullus vulgaris</i> Schader, <i>Cucumis</i> spp., <i>Cucurbita pepo</i> Linnaeus, varieties and <i>Echollium elaterium</i> Rich.	—	2 to 3 days in summer; 7 in autumn.	8 to 18 days; larvae burrow into fruits where they feed on seeds.	Passes winter as pupa in soil; normally pupal stage lasts 13 to 20 days or more; in Saudi Arabia about 6 generations per year with much overlapping.	Emergence begins in June in Transcaucasia, continuing for about 3 weeks.	Each female lays at least 100 eggs in pulp of fruit; preferably in newly set fruits.
<i>Platypharea pocitoptera</i> (Schrank) (Asparagus fly). Central and southern part of Europe.	Asparagus only known cultivated host.	—	Few days.	3 to 4 weeks.	Overwinter as pupae in asparagus stems with extended period of emergence.	—	Deposited singly; as many as 20 larvae and pupae in one shoot.
<i>Rhagoletis cerasi</i> (Linnaeus) (European cherry fruit fly). Continental Europe and parts of Turkey and Iran in Asia; in U.S.S.R. extends from southern Leningrad Province to the Crimea and into western Kazakhstan.	Serious pest of cherries but attacks other species of <i>Prunus</i> ; species of <i>Loniceta</i> appear to be important alternate hosts.	After about 11 days begin laying eggs.	6 to 12 days.	30 days.	From formation until following May; sometimes spend 2 winters in soil.	In Norway adults present on cherry trees about 50 days.	50 to 60 with maximum of 100; usually 1 per fruit.
<i>Rhagoletis completa</i> Cresson (Walnut husk fly). Western United States (14).	All <i>Juglans</i> spp. all varieties. <i>Juglans regia</i> Linnaeus (Persian walnut) (14); peach, first eggs available under conditions of artificial food; average 18 to 24 days; field conditions natural food (14).	Mating 7 to 14 days average field conditions (14); first eggs available under conditions of artificial food; average 18 to 24 days; field conditions natural food (14).	5 days. conditions	45–104°F. (mean 64°F.) 36.8 days; 67–90°F. (mean 82°F.) 27.9 days (14).	291 to 328 days (14).	Most favorable laboratory conditions (virgin females average 35 days; field conditions 30 to 40 days (14)).	200 to 400 eggs, summer conditions Calif
<i>Rhagoletis pomonella</i> (Walsh) (Apple maggot, bittersweet, etc.). Mainly northeast U.S. and southeast Canada; as far west as N. Dak. and southward to Ohio (47); more abundant in northern part of range.	Apples, crab apples, pears, plums, bittersweet, etc. and huckleberries (47).	In cages without flies 14 to 31 days; average 20 days; yeast hydrolysate 8 days; average 75°F. (37a).	3 to 7 days (47); 2 to 8 days (35). Minimum 5 days. Average 4.5 days. Mean temperature 75°F. (37a).	Minimum of 2 weeks in early applies to winter applies (47). 20 to 22 days in summer; longer toward fall (35).	Most puparia remain in soil over 1 year; average summer; few remain over 2 years (47) and a very small number 3 or 4 yr. (35). Thus pupal period could be 11 to 44 mos. in Maine; N. J. (37a). Maximum of 60 days in cages (20).	Caged female in summer (47) to 24 days in field in summer in Maine [data by curve fitting (35)]. Average in lab tests a maximum of 277; more eggs obtained from rich protein diets (20).	Potential of 288 eggs based on dissection (30); potential of 300 to 400 on basis of examination of ovaries. In lab tests a maximum of 277; more eggs obtained from rich protein diets (20).

which is spent buried in the soil, but it is possible that light stimuli operating on other stages or on the plant host may determine the occurrence and nature of diapause in pupae. When adults of *D. tryoni* were kept in certain cycles of light and darkness in tests conducted by Bateman (10), the pupae of their offspring had rhythms of ecdysis different from the controls. The same cycles applied directly to the pupae had no such effect. The relationship of temperature to diapause as discussed by Boyce (13) and the role of other physical, biochemical, or genetic factors in stimulating or breaking diapause in fruit flies are in urgent need of critical investigation.

BIOLOGICAL RACES

A number of unusually challenging biological race problems are associated with fruit flies.

A number of American workers have indicated that *R. pomonella* is one of several populations of North American fruit flies with similar morphological features. For instance, *zephyria* Snow is a *Rhagoletis* whose differences from *pomonella* are very slight; *Rhagoletis symphoricarpi* Curran from snowberry is a closely related form; and *Rhagoletis mendax* Curran from blueberry has much the same type of habitus. Various writers, among whom Aldrich, Benjamin, and Curran figure prominently, have synonymized one or more of these forms with *R. pomonella* and with each other on morphological grounds. Pickett (46) examined "*pomonella*" reared from apple, hawthorn, and blueberry, and, on the basis of certain features of wing pattern and penis, concluded that all forms are representatives of a single species.

Biologically *R. pomonella* has been regarded as monophagous, with strains or forms adapted to specific fruits, but the evidence has been rather conflicting. Lathrop & Nickels (34) reared *R. pomonella* from nine species of plants growing in association with blueberries in Maine and concluded that the forms in apple and blueberry are distinct and independent ecologically. Illingworth (29) gave an account of the hosts known in 1912 but without conclusions as to the status of the various forms. Woods (62), by making various transfers of larvae from one host to another, concluded that the apple and blueberry forms were "independent," a conclusion also reached by Patch & Woods (44). Porter (47) also expressed doubt that the flies infesting the various hosts were all of the same species.

On the other hand, McAlister & Anderson (39) obtained successful crossings of females reared from apples with males from blueberry, with viable F₁ progeny, but reciprocal crosses were less successful. Pickett (46) obtained conflicting results in extensive crossbreeding work but, after a long association with, and careful observations of, various forms, concluded that "*pomonella*" in the broad sense is an oligophagous fruit fly with populations in hosts not sufficiently distinct to warrant calling them species. Pickett & Neary (45) further concluded that hosts have a definite effect on the seasonal history of these fruit flies, those adapted to one host reacting differently from those adapted to another. Hall (27) concluded, on the basis of numerous

crossbreeding experiments, that *R. pomonella* in dogwood in Ontario is a distinct biological race which will not interbreed with the race from apple and hawthorn. The problem of host races or host subspecies is an intricate one, and *R. pomonella* appears to present promising material for further investigations.

Rhagoletis cingulata (Loew) was described by Loew in 1862 as being from the "middle states." Since the early 1920's, *cingulata*-like flies have been known to attack cultivated cherries in the Pacific Northwest and were generally assumed to have been introduced from the eastern states. Observations by S. C. Jones in 1930 indicated that populations of a close relative of *R. cingulata* on bitter cherry (*Prunus emarginata* Walpers) were reluctant to attack nearby cultivated cherry. This pest of wild cherry was described by Curran as a new species, *Rhagoletis indifferens* Curran, on the basis of male terminalia. Curran stated that oviposition of *R. indifferens* had not been induced upon cultivated cherry, nor had *R. cingulata* been induced to oviposit on wild cherry. Benjamin (11), from his observations on the wide variation in the terminalia characters among individuals of *R. cingulata* reared from a single host, considered *R. indifferens* to be *R. cingulata*, and this conclusion was supported by later writers. Simkover (55) also found wide variation in the terminalia characters.

In 1942 and 1943, Jones (31) produced fertile offspring by interbreeding the cherry fruit flies from cultivated and wild cherry. Moreover, he succeeded in obtaining normal adults by transferring larvae from one host to another and inducing flies from bitter cherry to oviposit in cultivated cherries and vice versa. He concluded that there were no significant taxonomic differences between the two populations.

Simkover (55) collected *cingulata*-like pupae from beneath *P. emarginata* at White Salmon, Washington, the type locality of *R. indifferens*, and pupae from beneath Bing cherry trees at Prosser, Washington, a location isolated from wild cherries. When crossed both ways, adults from these two populations showed perfect interfertility in the F_1 generation. There were no significant differences in width of ejaculatory apodemes or in time of emergence. The F_2 generations likewise appeared to interbreed readily. When offered freedom of choice between cultivated and wild cherry in which to oviposit, all crosses distinctly preferred cultivated cherry, regardless of the former host.

Blanc & Keifer (12) became interested in searching for differences that might exist between wild and cultivated cherry *R. cingulata* of the western states and typical *R. cingulata* described by Loew from the eastern states. After studying large amounts of material from numerous localities, they concluded that differences in a number of characters were constant and that populations from the two regions could always be separated in the larvae by characteristics of the thoracic spiracle and postspiracular slits and in the adult by fuscous markings near the wing tip, color of anterior coxae, apical markings of abdominal tergites, and the length-to-width ratio of the female

ovipositor. They also found remarkable correspondence in distribution between *R. indifferens* and its wild cherry host (*P. emarginata*) in the West, and between the wild black cherry (*Prunus serotina* Ehrhart) and typical *R. cingulata* in the midwestern and eastern states. Distributions of these two plants are allopatric.

By the use of a discrimination grid [Mayr, Linsley & Usinger, p. 79 (38)], Blanc & Keifer (12) concluded that the two fruit-fly populations were either different species or subspecies, and, not being able to test the degree of reproductive isolation, they elected to call the eastern form *Rhagoletis cingulata cingulata* (Loew) and the western one *Rhagoletis cingulata indifferens* Curran. Blanc & Keifer (12) mentioned also the possibility that yet other populations of *R. cingulata* might exist in certain areas of California. They indicated that length-to-width ratios of posterior spiracular openings in larvae collected at Dutch Flat in the Sierra Nevada Mountains differ significantly from those occurring farther north.

Anastrepha fraterculus (Wiedemann), originally described in Brazil, is a fruit fly of considerable economic importance in South America, where it has been reported from at least 22 hosts. Grapes, peaches, and guavas are particularly subject to attack. Work in Mexico by Baker *et al.* (7) indicated that certain flies resembling *A. fraterculus* in Veracruz, Morelos, Tamaulipas, and Coahuila differ in several important respects from South American *A. fraterculus* known to them. The so-called "V-band" of South American *A. fraterculus* is usually separated from the S-band, while there is normally a connection between the two in Mexican *A. fraterculus*. According to Baker *et al.* (7), although these bands may be separated in one wing of the Mexican form, the other wing customarily retains the connection, and, when a separation does occur, it is usually in the male. The ovipositor tip of Mexican *A. fraterculus* seems to be somewhat more tapered.

Host differences in the two forms are rather striking. Mexican *A. fraterculus* has been found in the field only in peach, guava, rose apple, and tropical almond, and, unlike the South American form, has never been observed in citrus plantings in immediate proximity to these hosts. Baker (9) found the Mexican form to oviposit in the laboratory on jobo, pear, loquat, plum, grape, and coffee berries, which are normal hosts for South American *A. fraterculus*, but obtained no larval development in any of these hosts, nor did he find oviposition on citrus, mango, or cherimoya in the laboratory. Grapes proved to be an excellent egg-capturing medium, since Mexican *A. fraterculus* deposits large numbers of eggs in this fruit; yet no larval development was ever observed in it. Brazilian *A. fraterculus* is an important pest of the grape industry.

Mexican *A. fraterculus* has been found in rose apple only in the vicinity of Cordoba, Veracruz. Baker *et al.* (7) compared the response to temperature of development of eggs on this host with that of *A. ludens*, *Anastrepha serpentina* (Wiedemann), and *Anastrepha mombinpraecoptans* Sein [Baker (8)]. When the reciprocal or egg-development time was plotted against

temperature, separate lines were obtained. If experiments with Mexican *A. fraterculus* from guava and peaches and with South American *A. fraterculus* can ever be conducted under comparable conditions, they may provide additional biological factors for separating these two populations.

Disagreeing with the conclusions of Baker *et al.* (7), Stone (61) considered specimens of *A. fraterculus* with wing bands connected to be no more than a "geographical race," and believed that *A. fraterculus* is a variable species extending from the Rio Grande Valley to Argentina and Chile. The true status of Mexican, Brazilian, and possibly other segregates of *A. fraterculus* may not be satisfactorily decided until they can be brought together for crossbreeding studies.

A. mombinpraeoptans is an important fruit fly in Mexico and in much of the West Indies. References to this fruit fly under the name "*acidusa*" are common in the early literature, and in early reports the Mexican form is referred to as both "*acidusa*" and "*fraterculus*." In the opinion of Baker *et al.* (7), Mexican *A. mombinpraeoptans* differs significantly from the species in Puerto Rico, although Stone (61) stated that both populations are of the same species. Baker *et al.* (7) noted differences in wing patterns of adults and in certain features of posterior larval spiracles that appear to be correlated with differences in hosts.

In Puerto Rico, *A. mombinpraeoptans* is an important pest of mango, but in Morelos, Mexico, it prefers *Spondias* even when the two fruits are growing side by side. It has also refused mangoes heavily infested with *A. ludens* larvae in laboratory cages in Mexico. In Veracruz, mangoes are infested to some extent. It would be of interest to determine the differences, if any, between this mango feeder and the Puerto Rico fruit fly and also between Mexican and West Indian populations, regardless of host.

Adults from an *A. mombinpraeoptans*-*Anastrepha suspensa* Loew cross have been obtained at Key West, Florida. Stone (61) regarded the characteristics of these adults as being intermediate between the two species, and, never having seen field-collected specimens resembling them, suggested that such crossing may never actually occur in nature. Also of interest are crossbreeding experiments conducted in Panama by J. Zetek involving *A. mombinpraeoptans* and *A. fraterculus* (61a). Unfortunately, the experiments were apparently initiated under conditions that did not assure complete segregation of the two species, and at the end of the series only *A. mombinpraeoptans* remained. In private communication, A. Stone has indicated extreme doubt whether productive crossing between these two species is possible under any conditions.

PARASITISM AND SYMBIOTES

Fruit flies are attacked by a variety of opiine and other parasites as well as predators. Several explorations have been undertaken to find and introduce beneficial insects into different parts of the world where especially damaging fruit-fly species occur. The effectiveness of these biological control efforts have been evaluated by Clausen (17).

Bacteria and other microorganisms are known to occur in all stages of fruit flies. Their relationships to physiological processes, especially the extent to which they may produce vitamins and growth-accessory materials, stimulate or regulate egg hatch or other metamorphic events, aid in digestion of foods, or be indispensable to development in other ways, offer fascinating opportunities for productive research.

A bacterium, *Ascobacterium luteum* Babes, may occur in all stages of the olive fly, *Dacus oleae* (Gmelin). Petri suggested [see Steinhaus (60)] that the lipase produced by the bacterium aids in the digestion of olive tissue, rich in oil, consumed by the larva, but later he found that not all larvae contain the bacterium. Apparently of much significance are the structural modifications and processes in the olive fly which insure transmission of this bacterium through all stages. As eggs pass along the vagina, their surfaces are pressed through a slit against the openings of saclike evaginations in the wall of the anal tract which is filled with the bacteria. The latter are thus smeared over the egg surfaces, and then find their way through the micropyle into the egg, where they invade the embryo. In the larva, the bacteria occur in four special caeca near the forepart of the mid-gut and also throughout the alimentary tract. In the pupal stage, bulblike diverticula from the oesophagus accommodate them, and from there they spread throughout the alimentary tract.

During the first successful *C. capitata* eradication campaign in Florida in 1929 and 1930, the theory was developed that the toxicity of copper carbonate results from its action on the fruit fly's intestinal flora [Baker *et al.* (7)]. Later Plummer developed a combination copper and sugar fruit-fly insecticide known as copper sucrose [U. S. Pat. 2,138,557 (1938)], which proved to be slow in its action but was promising in tests with *A. mombin-praeoptans*, *A. ludens*, and *Anastrepha striata* Shiner in Mexico and especially so against *C. capitata* in Hawaii in tests conducted by McBride & Marlowe [Baker *et al.* (7)].

The possibility of controlling fruit flies with substances that affect their intestinal flora deserves additional study. If microorganisms are indispensable to the longevity and reproductivity of fruit flies, the attritional effect of antibiotics introduced into their environments as food contaminants would be interesting to observe. The many new biological materials now available to supplement synthetic compounds known to have bactericidal or fungicidal action would increase the significance of this experimentation. In addition to their application in sprays, the antibiotics might also be applied in combination with protein hydrolysate nutrients, which the flies eat avidly.

MOVEMENT

Most fruit flies are strong and vigorous flyers. Long distances may be covered within a few days by especially active species. The males of *D. dorsalis* have frequently been found to travel 4 to 15 mi. from the point of their release and to cross 9 mi. of open sea between islands. Occasionally this

species may fly several miles within a few days (61a). Presumably as a result of long range flight, each fall and winter *A. ludens* invade citrus groves in south Texas, approximately 80 airline miles from the nearest known breeding area in Mexico. Occasionally individuals have been trapped as far north as Falfurias and Dimmit, Texas, approximately 160 and 175 mi. respectively, from the nearest breeding area (data from studies made by N. O. Berry and associates, Plant Pest Control Division, U. S. Department of Agriculture).

Marked *C. capitata* flies released in Hawaii by Severin & Hartung (53) in 1912 were recovered from $\frac{1}{4}$ to $1\frac{1}{2}$ mi. from the release point. In recent tests conducted by Steiner, flights of nearly 2 mi. have been recorded, and 7 per cent of 944 marked flies released in a large Macadamia nut grove were recovered in traps from $\frac{3}{4}$ to $1\frac{1}{2}$ mi. away, the farthest traps in this experiment. A naturally emerging male fly tagged with P_{32} was recovered more than 20 mi. away, with the distance traversed including at least 9 mi. or more of open sea. The frequency with which the Mediterranean fruit fly has been caught more than a mile from its release suggests that many may go much further, especially when generally unfavorable host conditions prevail.

The weather strongly influences fruit-fly movement. A correlation between wind direction and direction of flight of *C. capitata* was noted in the marked-fly tests reported in 1912 by Severin & Hartung (53) and in more extensive recent tests conducted by Steiner (61a). In the Kat River Valley of the Union of South Africa, Ripley *et al.* (49) were unable to detect any relationship between wind and direction of *C. capitata* flight, but the erratic local air movement made it difficult to arrive at conclusions. In other tests in Hawaii, Henderson and Keiser (61a) and Gammon (California Department of Agriculture) noted an effect of wind on direction of *D. dorsalis* flight, but many marked individuals made their way for long distances against the prevailing air flow.

The influence of air movement on fruit-fly flight does not appear to be an obligatory one, at least when low to moderate velocities are involved. Males of *D. dorsalis* make their way readily to methyl eugenol traps against strong trade winds. Normal orientation of fruit flies to hosts requires flight against air movement, and in Hawaii, at least, *D. dorsalis* adults readily find and infest favorable hosts regardless of their location with reference to trade-wind patterns.

The relationship of high-velocity winds, such as those provided by local storms, hurricanes, or typhoons, to the distribution of fruit flies can only be speculated. Undoubtedly, such winds pick up many objects as small as fruit flies, and presumably they can carry and deposit them at locations many miles away. Differences in the insect faunas of islands and mainland areas in the paths of hurricanes or typhoons, together with the seeming association between the movement of man and infested fruits and vegetables in travel and commerce and the long-range movement of fruit flies, suggest that establishment may be an infrequent result if high-velocity windborne introductions are of common occurrence.

Fruit flies are generally inactive at night and during periods of moderate to heavy rainfall. Movement and orientation in response to the fruiting or ripening of favorable hosts is a well-known common attribute of species which have been studied. Ripley *et al.* (49) noted minor mass movements of *C. capitata* which they attributed to irregularity in the distribution of honeydew food. They also observed the greatest activity and mass movement on hot, dry days in South Africa following dewless nights. The only climatic factor that could be correlated with the movement was low humidity. The need to replenish water losses under these conditions was suggested as the probable stimulus for such movement.

COMPETITION

One of the most interesting of fruit-fly relationships is the competition between species. Within a short time after *D. dorsalis* was found in Hawaii in 1946, *C. capitata* populations declined to such a degree that only rarely could adult flies or infested fruits be found in littoral areas. The Mediterranean fruit fly is now restricted almost entirely to cool climates at higher elevations, where it has a slight developmental advantage over the oriental fruit fly, or to coffee areas, where host differences may possibly cause it to be less affected by the presence of *D. dorsalis*.

Extremely light or rare infestations of *C. capitata* in Costa Rica in certain hosts usually preferred by this fruit fly also suggest competition from species of *Anastrepha*. Informal reports from Australia have indicated that *C. capitata* populations there may be curtailed by the presence of *D. tryoni*. The ability of *A. striata* in Mexico and Central America to dominate guava, a host favored by many fruit flies, is also suggestive of a competitive advantage. Competition from *D. dorsalis* and perhaps other fruit flies has been suggested to explain the failure of *C. capitata* to become established in southeastern Asia.

As a result of studies conducted in Hawaii, the dominance attained by *D. dorsalis* over *C. capitata* in Hawaii is now believed to accrue from the pronounced habit of the former of using *C. capitata* egg punctures for ovipositional purposes, and the inability of most *C. capitata* larvae hatching in egg cavities also inhabited by young *D. dorsalis* larvae to complete their development. When both fruit flies were allowed to oviposit in guavas and papayas, ultimate emergence was predominantly *D. dorsalis*, whereas *C. capitata* eggs in these fruits without *D. dorsalis* developed normally. Similar inhibition of *C. capitata* development occurred when large numbers of eggs of both species were placed together at the center of trays of carrot-rearing medium. However, when comparable numbers of *D. dorsalis* larvae newly hatched from eggs held separately were placed over newly hatched *C. capitata* larvae on the carrot medium, there was little or no evidence of competition. Depression of the *C. capitata* population in the rearing medium and inhibition of development in fruits were especially pronounced when *D. dorsalis* hatch preceded *C. capitata* hatch by 12 to 48 hr. When *C. capitata* larvae

were at least two days old before *D. dorsalis* eggs hatched, there was no significant inhibition of *C. capitata* development.

In nature, reduction of *C. capitata* populations to the point of virtual extinction by a strong lethal factor supplied by, or associated with, the presence of *D. dorsalis* would require more or less undiminished operation of the factor on a number of successive generations. The ability of *D. dorsalis* to locate promptly and deposit its own eggs in *C. capitata* egg punctures also appears to be a vital requirement. Although the numbers of *D. dorsalis* in Hawaii have been reduced by the introduction of biological control agents, the populations still existing in most areas have been adequate to prevent appreciable *C. capitata* reproduction.

Competition among fruit flies may be responsible for misleading impressions of host preferences. In recent years, intensive host surveys in littoral areas of Hawaii only rarely would have revealed *C. capitata* infestations in guava, mango, or other highly preferred hosts before the advent of *D. dorsalis*. This possibility that information on host preference may have been influenced by competition emphasizes the need for caution in the use of natural infestation data from different areas as a basis for designating biological races or strains. Furthermore, failure of a species of quarantine importance to cause natural infestation in a fruit or vegetable area where other fruit flies occur may not constitute reliable proof that the same fruit or vegetable could not serve as a host in other circumstances.

Research on competition among fruit flies may eventually suggest introduction of a species representing a lesser economic hazard to replace a more serious pest. Imposing obstacles to species manipulation such as this would appear to be the added quarantine involvements and the problem of bringing the different fruit flies together to develop a biological basis for such a program.

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HOST SELECTION IN PHYTOPHAGOUS INSECTS¹

By A. J. THORSTEINSON

Department of Entomology, University of Manitoba, Winnipeg, Canada

Flowering plants reputedly immune to attack by virtually all insects seem to be rarities. The chinaberry tree, *Melia azedarack* Linnaeus, is said to be such a curiosity [Painter (57)]. On the other hand, no plant species is attacked by all the insect species in its environment. Nor is it common, if it occurs at all, that an insect species devours indiscriminately all the plants in its geographic range. Many insect species are conspicuously associated with particular plant species as is evident from the common names of many insects (potato beetle, corn borer, etc.). Furthermore, each insect species is associated with a group of plants, large or small in number, which we designate as its food-plant range. The food-plant range of some insects is curiously correlated with natural taxonomic plant groupings (genera or families, etc.), but the food plants of many insects are distributed in an apparently random pattern among plants without special regard to botanical affinities.

These considerations clearly imply the existence of mechanisms that participate in the effective allocation of grazing privileges to herbivorous insects. The outcome of this allocation yields the patterns of "host-plant selection" or "food-plant preferences" that we observe among phytophagous insects.

The terms "selection" and "preference" are necessarily anthropocentric, but it should be permissible to retain them until more objective, yet equally convenient, terms are coined. It is, however, necessary to define as precisely as possible what we mean when we use these terms in the context of insect behaviour.

A phytophagous insect in the presence of a plant located either through its own foraging activities or otherwise (for example, having hatched thereon from an egg deposited by its mother) either does or does not feed on the plant. If it does not feed, and its powers of locomotion are unequal to the task of seeking food elsewhere, it must die of starvation, thereby indicting its mother biologically for an error of judgement and simultaneously weeding out the aberrant genetic tendencies that would otherwise disintegrate the relatively stereotyped food plant association patterns of its species.

On the other hand, if the insect is sufficiently mobile, it has the alternative of foraging elsewhere in search of a more suitable plant. Indeed, alate insects may also abandon their preferred food plants after a period of feeding activity, ostensibly in the interests of dispersal. This apparently incongruous behaviour becomes entirely reconcilable with the more summary departures, not only teleologically but also mechanistically, when we discuss the counter-

¹ The survey of the literature pertaining to this review was concluded in March, 1959.

poised neuroregulatory systems concerned with feeding and dispersal behaviour.

In any event, food-plant selection consists essentially of a series of take-it-or-leave-it situations in which the insect either accepts or rejects the plant at hand as food for itself or its offspring. This is not to say that feeding or oviposition behaviour is an all-or-nothing phenomenon. Feeding may continue only through the duration of the initially lowest thresholds of response; should the plant contain a feeding inhibitor, e.g. demissin, the inhibitor's restraining influence may presently prevail as the thresholds for feeding stimuli rise, as is graphically depicted by de Wilde (80). There is no need to postulate anything like "comparison shopping" to account for our observations of food selection by insects—a more mechanical model seems to be adequate. The aggregation of individuals of a given insect species on particular plants is the statistical end result of a higher frequency of visitation on the one hand and longer duration of sojourn on the other. The conditions that influence and the mechanisms that regulate these two phenomena constitute the field of insect biology designated by the term "food-plant selection."

The literature specifically concerned with the critical experimental investigation of food-plant selection is not voluminous. One can only speculate as to the reasons for this slow development. These might include difficulties of experimental technique, lack of relevant knowledge of plant chemistry, inadequate insight into the complexities of food finding, oviposition, and feeding behaviour of insects, and, perhaps, a premature generalization from the early, essentially successful solution of a special case of the problem by Vershaffelt (73).

These obstacles are now being overcome and an increased rate of discovery is supplanting the antecedent speculations and theories that served, at least, to manifest the interest of biologists in the problem and to stimulate investigation.

The first volume in these reviews included an account, together with an exhaustive bibliography, of food-plant selection as an aspect of insect nutrition [Lipke & Fraenkel (45)], which was supplemented by Friend (26). A concise outline was provided by de Wilde (79). Chauvin (8) discussed food-plant selections as an aspect of insect behaviour. Dethier's (15) *Chemical Insect Attractants and Repellents* remains a treasury of information. Martin (48) has reviewed the chemical defences of plants against insects. The survey by Kuznetsov (42) and other relevant Russian literature were not available in readable form to this reviewer at the time of writing.

A most auspicious development favouring progress in the investigation of food-plant selection is the series of symposia on insect-plant relationships that began in Amsterdam in 1952, followed by a second in Wageningen, 1957, the third at Detroit, 1959, and a projected fourth in Vienna, 1960. Papers presented in the second symposium were published collectively in the first number of the new international journal, *Entomologia Experimentalis et Applicata*, Amsterdam. A paper not purportedly concerned with food-plant

selection as such, but of fundamental significance both in design of experiments and interpretation of data, is that of Kennedy (39). Other important contributions are referred to herein in the appropriate contexts.

FOOD-PLANT PERCEPTION

Recent reviews of our knowledge of food-plant perception of insects have been provided by Dethier (16) and de Wilde (79). These valuable accounts have been followed by new discoveries and insights into this problem.

The overt behaviour that relates directly or indirectly to the food-plant relationships of a phytophagous insect appears to comprise two quite distinct, alternating phases: dispersal and actual feeding. Inasmuch as phytophagous insects cannot engage in both these activities simultaneously, the two neuromuscular systems serving locomotion and ingestion appear to be inversely, even antagonistically, related to the central neuroregulatory centres that control excitation thresholds for external stimuli. Excellent illustrations of the alternating dominance of these two behavioural regimes are provided in the biology of alate aphids by Kennedy (39) and of *Leptinotarsa decemlineata* (Say) larvae by de Wilde (80). A most remarkable illustration of the dominance of dispersal stimuli over food-plant colonization in aphids is reported by Müller (50). It seems probable that this may be the fundamental pattern on which all the variations in insect-plant behavioural relations are superimposed.

Kennedy (39) proposed the attractive hypothesis that, during the dispersal flight, the thresholds of stimuli generated or reflected by the food plant are lowered to a level at which the insect becomes responsive to these chemotactic and electromagnetic signals. The probability is thereby increased that the insect will alight on a food plant in the vicinity and "recognize" (that is, remain on) it. Before this goal is achieved, the thresholds for stimuli, such as light and heat, that excite dispersal activity are rising to a level at which these stimuli do not effectively conflict with those originating from the host plant. The concept that consummation of one stage in a series of behavioural phases in some way facilitates responsiveness of the animal to stimuli that excite the next stage had been recognized previously, but its significance for reconciliation of feeding and dispersal behaviour was not appreciated. Kennedy (39) coined the term "priming" for this effect and revived the concept advanced earlier by Sherrington that, in addition to excitation of a given response, a stimulus also typically inhibits other responses. This principle is quite in harmony with, and is actually necessary to, the integrity of the basic pattern outlined above.

FOOD-PLANT FINDING

The problem of food finding was solved relatively early for wireworm larvae [Thorp *et al.* (65)], although the dense environment of wireworms would hardly facilitate either foraging or the transmission of chemotactic signals from the plant. It is strange that only limited success has been

achieved in the study of food-finding orientation in alate insects, although one inclines to assume that they possess delicately tuned navigational faculties together with efficient mechanisms for aeronautic locomotion.

On critical consideration one must conclude that none of the signals emanating from a single plant, whether they be visual or chemotactic, are likely to survive attenuation, for instance, at a distance of one kilometre, at intensities above the perception thresholds of insects. There may be exceptions to this generalization but, to my knowledge, there is no unequivocal evidence to contradict it. Visual signals, except at close range, may be relegated to a collateral role on the grounds that they do not contribute significantly to unique identification of food plants by insects. Olfactory signals are generally credited with a major role in food-finding mechanisms but clear evidence of their precise significance is scarce even for short-range perception of food plants. The "screen tests" of Dethier (11) and the "tube tests" of Munakata *et al.* (51) have demonstrated that larvae may perceive food plants at a range of several millimetres. Schwink (61) has shown that the perception of sexual attractants in moths operates only over a few decimetres. There seems to be no critical evidence that insects orientate to plants beyond a few metres. This may be a result of the special experimental difficulties in such studies, but it is entirely plausible that foraging is random (not orientated) until the insect encounters the immediate vicinity of the food plant. It is indeed probable that some insects are unresponsive even to high intensities of their food-plant odors during the initial phase of the dispersal flight. Trouvelot (72) states there is no evidence that *L. decemlineata* adults are attracted to their food plants from a distance.

Many attempts have been made to study this problem through the use of olfactometres of diverse designs but often with sorry results. These disappointments might be ascribed to the "unnatural" restrictions imposed by the apparatus on freedom of movement or the introduction of extraneous, thigmotactic stimuli. More probably, failure has resulted from a lack of insight into the interacting influences of external stimuli in rapport with waxing and waning threshold levels involved in the counterpoised behavioural phases of feeding and dispersal.

The relatively small number of demonstrated attractants of botanical origin for phytophagous insects have been noted by Dethier (15). These examples of attraction are of interest, but they do not, insofar as we can see at present, decisively explain the host-selection behaviour of the insects concerned. In view of the presumed importance of olfaction in food-plant selection, it appears justifiable to establish the criterion that the attractant odor be derived from a substance which is generally peculiar to the food plants of the insects. The demonstration of this ideal relationship has recently been achieved in the case of the attractance of coumarin for the sweet clover weevil, *Sitona cylindricollis* (Fähræus) [Thorsteinson *et al.* (70)].

The influence of coumarin on the sweet clover weevil cannot readily be

demonstrated in the laboratory because, apparently, the insect is responsive to plant odor only on the wing during the dispersal flight; this is in harmony with the concept of Kennedy (39). For this reason, it appears that a study of flight behaviour is necessary to the understanding of food-finding mechanisms of alate, phytophagous insects. Such investigations have been made for several insects [Hans (30); Kennedy (39); Le Berre (43, 44)]. Because of the apparent intimate association between olfactory response and flight activity, it might be advantageous to demonstrate chemotactic homing on food plants in the field by means of suitable traps. Olfactometric laboratory arrangements would require designs incorporating provision for observing behaviour during flight.

Another serious obstacle in such studies is our limited knowledge of the chemistry of plant odors. Of course, many essential oils and other aromatic substances of botanical origin have been identified. The fact that coumarin, the source of the characteristic odor of *Melilotus* spp., is also an article of commerce in the perfume industry and is, in any case, very easily prepared from the leaves of the sweet clover plant facilitated the study of its olfactory influence on *S. cylindricollis* [Thorsteinson *et al.* (70)]. It may be remarked here that, although coumarin is undoubtedly a significant cue to food-plant recognition, there is nothing to suggest that it draws weevils from any appreciable distance, nor does it seem to be a necessary feeding stimulant. The effective concentration of coumarin in weevil traps is high enough to be easily perceived by the human sense of smell at a distance of one metre.

Some plants susceptible to oligophagous insects, such as the potato, do not contain recognized odor substances easily isolated or otherwise available for experimental use. A haunting apprehension is that insects may perceive odors to which the human olfactory sense is not attuned, thus adding a peculiarly frustrating difficulty to the other obstacles confronting the experimentalist. Although this may be true, it would be well to bear in mind that where a human cannot perceive an odor, perhaps the insect also cannot perceive any that uniquely identifies its food plant. That is to say, the insect may respond by olfaction, if at all, only to plant odors of rather general botanical distribution and find its food plant less by attraction thereto than by avoidance of other plants, the odors of which are presumed to be repellent. As we shall see presently, this principle of host avoidance [named such by Wardle (75)] is assuming new significance in the explanation of feeding behaviour and is equally tenable in regard to both olfactory and gustatory manifestations of the chemotactic regulation of food-plant preferences.

Whether attractant odors really function as such, in the sense of drawing insects from a distance along an odor gradient, is not established. Other factors probably must contribute to such an orientation, e.g. anemotaxis, which in turn must involve visual orientation to ground patterns [Kennedy (40)], and calibration of a metabolic energy metre. Wright (83) has speculated that odor pulse frequencies alone could provide adequate orientation.

A difficulty in these theories is that the odor is originally expected to excite locomotory activity and later (at the food plant) to participate in inhibiting locomotion. This is not an irreconcilable contradiction, but there is little or no evidence concerning the initial excitatory influence of odors presumed to be attractive, and other influences (heat and light) are known to be perfectly adequate to stimulate flight in several insects, even inciting them to leave their preferred food plants where the olfactory stimulus is high. On the other hand, there can be no question that successful dispersal terminates at loci where the stimuli provided by the food plants are high, and, if these include an olfactory stimulus, it is at least compatible with cessation of locomotory activity.

This reviewer suggests that a principal effect of odors emanating from food plants is to inhibit locomotory activity (negative chemokinesis) when olfactory thresholds have fallen sufficiently during dispersal movement. This would adequately explain most of the observed insect aggregations on their food plants, and a chemical that has this effect would constitute an "attractant" in the loose sense of the word although another term, say "aggregant," might be more appropriate. If this is true, the influence of odors on insects approximates that of sugars as explained by Dethier (19), except that the insect becomes "aware" of the food at a short distance from it and need not blunder into actual contact with it before beginning to "recognize" it.

FOOD-PLANT RECOGNITION

Recognition of the food plant may overlap, in part, the final stage of the food-finding phase as suggested above, but the decisive manifestation of food-plant recognition is the deferment of renewed dispersal activity for an indefinite period during which feeding or oviposition, or both, proceed at a rate favouring survival and increase. This outcome necessarily implies: (a) thresholds for dispersal stimuli remain sufficiently high to preclude wanderlust, (b) thresholds for feeding stimuli remain sufficiently low to favour feeding activity, (c) the plant possesses the attributes that supply all the required sorts of feeding stimuli, and (d) there are no repellents or inhibitors of feeding or oviposition. It is now necessary to identify what plant attributes are effective promoters of feeding and to evaluate the relative importance of these attributes.

PERMISSIVE FACTORS

Succulence, toughness, pilosity, and so forth, are usually not to be regarded as stimuli, but they must be taken into account. Where they present obstacles to the feeding mechanism, they tend to preclude a response to the feeding stimuli. Resistance of plants to insects has come to be uncritically associated with these morphological attributes of plants, but thorough investigations generally reveal that sensory or nutritional factors are also involved [Painter (57)].

TACTILE STIMULI

By definition, gustation is contact chemoreception and is inevitably associated with a tactile component. It can hardly be determined whether these associated tactile stimuli contribute significantly to the total signal input required to elicit an appreciable feeding response. It is known that some insects respond to sucrose, for example, in solution, although their natural food substrate more nearly approximates a gel. It appears certain that, without the modulating influence of chemotactic signals, the tactile stimuli do not normally command a feeding response in phytophagous insects. Observations of feeding, for example on filter paper, seldom exclude the possibility of a response to impurities or moisture, and, in any event, they typically occur under conditions of impending inanition when chemotactic thresholds have presumably dropped to a very low level. Even so, the rate and volume of response hardly compares with that to a preferred food plant.

The influence of tactile stimuli on oviposition behaviour appears much more significant. For example, the diamondback moth, *Plutella maculipennis* (Curtis), lays eggs much more freely on a pebbled, grooved, or pitted polyethylene surface than on a smooth substrate of the same material even if there is no olfactory stimulus [Gupta & Thorsteinson (29)]. However, coating the relief surface with mustard-leaf juice or pure allyl isothiocyanate at an optimum concentration further increases the deposition of eggs. The total oviposition response comprises a summation of the effects of tactile and chemotactic stimuli. The distribution of diamondback moth eggs on cabbage and on mustard plants differs, and the divergence is explicable in terms of these tactile and chemotactic effects.

VISUAL STIMULI

At least some phytophagous insects can perceive shapes with sufficient acuity to suggest that plant silhouettes contribute to the orientation mechanisms of food-finding, as for example, in the desert locust, *Schistocerca gregaria* (Forskål) [Wallace (74)]. However, silhouette cues can provide only a most tentative appraisal, if any, of acceptability of food plants.

The spectral composition of light reflected from the surfaces of plants is relatively narrow in range. Nevertheless, some phytophagous insects can perceive certain color differences [de Wilde & Pet (81)]. Since the leaves of most plants are some shade of green or yellow-green, it does not appear probable that color of plants can provide critical cues for the decisive recognition of preferred food plants. However, the color of plants does appear to play a role in food-plant finding. Yellow trays containing a nonrepellent fluid are efficient field traps for aphids [Moericke (49)]. The weevil, *Sitona lineata* (Linnaeus), is attracted to yellow and green traps, but white traps are also effective [Hans (30)]. The weevil, *S. cylindricollis*, is attracted appreciably more to yellow than red or blue traps, but the best success requires that the yellow traps be charged with coumarin, the principal odor substance of the food plant [Thorsteinson *et al.* (70)].

CHEMOTACTIC STIMULI

Although plants differ significantly in the physical attributes discussed above, none of these considered, either separately or together, provide a theoretical substrate adequate to account for the multifarious association patterns between insects and plants. The definitiveness of these associations demands the assumption that the attributes of plants present stimulus mosaics perceptually significant for insects. Shape, size, and color are too variable and lack the identifiable uniqueness required to explain the obvious discriminatory power of insects.

On the other hand, the chemical constitution of plants comprises an almost inexhaustible variety of substances. The permutations and combinations of these chemical constituents must greatly exceed any theoretical requirements for identification and recognition by insects adequate to the realization of food plant selection as we see it *fait accompli* in nature. One can hardly remain satisfied with these *a priori* considerations, however, unless it can be demonstrated that insects are, in fact, endowed with corresponding powers of delicate and discriminating chemotactic perception. Definitive confirmation of these faculties, as they relate to food plant selection has been slow in realization, but the rate of investigation in this field is accelerating. In addition to what has appeared in the literature, a full account requires reference to a number of recent unpublished findings in the author's laboratory.

OLFACTORY STIMULI

Odors are of significance in food-plant recognition during the final stage of food-plant finding as has already been discussed. Their role as actual feeding stimulants is inadequately established. Dethier (13) showed that certain essential oils induce *Papilio ajax* Linnaeus larvae to nibble on filter paper. However, mustard oil has only a very slight and uncertain effect on feeding responses in *P. maculipennis* larvae and coumarin does not increase feeding responses of *S. cylindricollis* adults [Thorsteinson *et al.* (70)]. Amputation of the antennae and palpi, the loci of the olfactory sensoria, in *L. decemlineata* larvae does not affect the quantity of acceptable leaves consumed but does increase feeding on leaves normally refused, suggesting that olfaction may be more concerned with avoidance than attraction [Chin (9)].

On the other hand, there are short-range influences, apparently attractive, of food-plant odors on locomotory activity of phytophagous larvae over distances of 1 or 2 cm. as demonstrated, for example, by Dethier (11), Grison (28), Munakata *et al.* (51), and Sugiyama & Matsumoto (63, 64). The observations of these authors appear to indicate the operation of some degree of attraction, but, in the opinion of this reviewer, a more significant function of odors for larvae is to inhibit locomotion away from the plant. In the event of accidental dislocation by wind, rain, or otherwise, visual stimuli (silhouettes) seem much better adapted to leading the larva back to its food plant than its very short-range olfactory perception.

The chemotactic influence of essential oils and other odorants.—No notice will be taken here of attractant or repellent effects of essential oils unless they be clearly interpretable in terms of food-plant selection. The *Melilotus* variety, Coumino, is attacked by blister beetles because of its lack of coumarin [Coplen (10)]. Coumarin, the odorous constituent of *Melilotus* spp., has been shown to help flying sweet clover weevils (*S. cylindricollis*) to find food plants [Thorsteinson *et al.* (70)]. An aliphatic unsaturated ketone, "Oryzanone," has been isolated and shown to attract or aggregate larvae of the rice stem borer, *Chilo suppressalis* (Walker) [Munakata *et al.* (51)].

Several essential oils in the food plants of *Papilio* larvae contribute to the stimuli that elicit feeding in these insects. These essential oils are not all chemically related, but their botanical distribution among the Umbelliferae and Rutaceae enabled Dethier (13) to trace a probable course of evolution of *Papilio* species in terms of the species' presently preferred food plants.

Mustard plants deprived of sulfur in hydroponic culture become so deficient in allyl isothiocyanate that it cannot be detected by the human chemical sense. The diamondback moth, *P. maculipennis*, tends to lay fewer eggs on such plants than on plants not so deprived [Thorsteinson *et al.* (70)]. Pure allyl isothiocyanate at higher concentrations inhibits egg deposition by this insect on plastic or clover leaf substrates. At lower (optimal) concentrations, egg deposition is increased well above the number laid on substrates devoid of mustard oil [Gupta & Thorsteinson (29)]. It is an interesting coincidence that allyl isothiocyanate, which regulates oviposition in the adult female *P. maculipennis*, is a hydrolytic fission product of sinigrin, which supplies a gustatory stimulus essential to feeding response in the larvae of the same species [Thorsteinson (66)]. The determination of food specificity is undoubtedly critical in the adult as well as in the larvae for many phytophagous species, but it does not follow that the effective chemical stimuli are identical. Free allyl isothiocyanate stimulates feeding by the larvae only to a very slight and nonessential extent.

The diamondback moth is regarded as an oligophagous insect and the mustard oils and their glucosides in this case might be conceived to be determinants of oligophagy. However, Sugiyama & Matusumoto (63, 64) have shown that mustard oils are attractants (aggregants) for the larvae of the vegetable weevil [*Listroderes costirostris obliquus* (Klug)] which is a polyphagous insect. The food-plant range of this insect includes both plants that contain mustard oils and many others that do not. This illustrates the complexities involved in correlating chemotactic influences with a simple classification of food-plant preferences; this correlation is the concern of a later section.

The chemotactic influence of water.—As pointed out by Fraenkel & Gunn (25) and Dethier (15), there is no reason not to consider hygroreception as a type of chemoreception. Behaviour of phytophagous insects in humidity gradients has been studied, for example, in the spruce budworm, *Choristoneura fumiferana* (Clemens) [Wellington (77)]. The anatomical location of

the hygroreceptors has been assigned to the antennae—the giant milkweed bug, *Oncopeltus fasciatus* (Dallas), serves as a phytophagous illustration [Anderson & Ball (1)]. Although water vapour is considered a regulating influence in insect behaviour, it does not seem to have been recognized as a significant factor in food-plant selection. This is understandable since the general impression is that a thin layer of moist air is associated with the surface of leaves and would furnish only an unspecific stimulus, if any. However, as I have previously suggested, the possibility that this hygrostimulus might inhibit dispersal in a manner similar to other more specific plant odors must be considered. In the absence of an inhibitory plant odor that would countermand it, the hygrostimulus alone might account for some of the reported responses of phytophagous insects to plant odors in olfactometers and "screen tests" where humidity effects may not have been taken into account. Dethier (11) clearly recognized this in his original screen tests. Hans (30) has shown that the weevil, *S. lineata*, in the ambulating state orientates to its leguminous host plants in an olfactometer according to their moisture contents. Grison (28) studied both water vapor and plant odor as separate influences in the behavior of *L. decemlineata*.

GUSTATORY STIMULI

Several studies currently in progress in the author's laboratory have yielded clear evidence for the possession by insects of a gustatory sense of unsuspected dimensions; this evidence offers considerable potential significance for a deeper understanding of insect-feeding behaviour. These studies as well as published information will be the subject of the following sections; the influence of constituents of plants will be considered, for convenience, according to their chemical classification.

The chemotactic influence of saccharides.—Gustatory perception of sugars in insects, especially haustellate types, has been discussed very thoroughly by Dethier (17). Frings (27) has remarked on the fact that many insects have a "sweet tooth." Beck (3) reported that the sugar content of the diet has a very marked effect on the establishment of *Pyrausta nubilalis* (Hübner) larvae on the nutritional substrate. Moderate concentrations of sucrose have a marked aggregating effect, correlating the distribution of *Pyrausta* larvae with sugar content of the various parts of the host plant. At higher concentrations beyond 0.03 M, the aggregating response declines, suggesting that very sweet plant tissues might not be attractive (reviewer's presumption, see below).

Insect responses to sucrose fall into a variety of patterns. For bees, flies, and butterflies, the feeding response to sucrose is proportional to the concentration, i.e. the modal response occurs at the highest concentrations obtainable—a behavioural adaptation that can only make these insects more efficient nectar foragers. The grasshopper, *Camnula pellucida* (Scudder), manifests an optimal response at about .02 M sucrose, fructose, glucose, maltose, and raffinose [Thorsteinson *et al.* (70)]. Nuorteva (52) reports that

leafhoppers avoid higher concentrations of sucrose. In view of the findings of Fraenkel (22) concerning toxic effects of sugar, these inhibitory effects of high sugar concentration may be a protective adaptation. Perhaps the resistance of sorghums to grasshoppers may be attributed in part to their high sugar content; grasshoppers also tend to prefer weeds to sugar-beet foliage, possibly for the same reason.

Further curious responses to sugars have been observed in our laboratory. *Plutella* larvae manifest negligible responses to sucrose at any concentration. Sinigrin alone elicits only a very weak response but 0.1 per cent sinigrin combined with 0.2 M sucrose evokes an appreciable feeding response. *Leptinotarsa* larvae respond well to sucrose concentrations of about 0.02 to 0.1 M but refuse fructose at any concentration [Thorsteinson *et al.* (70)]. *Choristoneura pinus* Freeman larvae accept certain sugars at concentrations of about 0.02 M [Heron (32)]. *Sitona* adults seem to be unresponsive to sugars except fructose which occasionally elicits a slight response [Thorsteinson *et al.* (70)]. In our laboratory, the grasshopper, *C. pellucida*, has been tested with a number of sugars. It is noteworthy that the sugars most stimulating to *Camnula* are almost exclusively those most abundant in wheat leaves—sucrose, fructose, glucose, and raffinose [Thorsteinson *et al.* (70)]. Since this relationship holds true not only for soluble saccharides as a group but also for the subgroups mono-, di-, and tri-saccharides, it seems unlikely to be entirely fortuitous and, teleologically, this is to be expected. Like the honey bee and unlike the blow fly [Dethier (17)], *Camnula* does not accept the indigestible sugar fucose. The silkworm, *Bombyx mori* (Linnaeus), will feed on an artificial diet devoid of mulberry leaves provided sucrose is added [Ito (34)].

In general, sugars appear to be of considerable significance in the regulation of feeding in phytophagous insects. As stated elsewhere in this section, in addition to their direct chemotactic influence, the saccharides interact as stimuli with several other groups of substances. The discovery and analysis of these interactions is a fruitful line of inquiry that will certainly add to our understanding of food-plant preferences. It is apparent, for example, that an insect which responds well to sugars at concentrations occurring in its food plant can hardly require a "secondary" or "odd" chemical stimulus to elicit feeding, and, therefore, such a special requirement is not likely to be involved in food selection insofar as feeding behaviour is concerned.

The chemotactic influence of organic nitrogen compounds.—Nuorteva (52) showed that albumin stimulates feeding in leafhoppers. Larvae of the house longhorn beetle, *Hylotrupes bajulus* (Linnaeus), feed best on diets containing peptone [Rasmussen (58)]. A striking influence of amides on the behaviour of wireworms was demonstrated by Thorpe *et al.* (65). Feeding responses of the grasshopper *Chorthippus longicornis* (Latrielle) to betaine, asparagine, and monosodium glutamate were reported by Thorsteinson (68). Beck & Hanec (4) found that several of the amino acids evoke an aggregating response in *Pyrausta* larvae. Several amino acids and amides evoke in *Camnula*

feeding responses which, although desultory, occur at rather low concentrations. A remarkable result is obtained when alanine, serine, and γ -aminobutyric acid are offered in mixture to *Camnula*; a very considerable response is evoked when each of these amino acids is present in the mixture at 0.0008 *M* concentration. This apparently synergistic effect is further increased by adding 0.02 *M* sucrose. Here again, the amino acids that participate in these interesting interactions are those found in the free state in greatest abundance in wheat-leaf extracts [Thorsteinson *et al.* (70)]. These active amino acids, however, do not evoke feeding at higher concentrations, e.g. 0.5 *M*, and under these conditions they must be considered as inhibitors. A number of the amino acids and amides elicit negligible feeding responses. It remains to be seen whether these are merely inert chemotactically or whether they are inhibitors of feeding. Auclair, Maltais & Cartier (2) have related aphid susceptibility in peas to certain amino acids in the food plants. The amino acids, γ -aminobutyric acid and alanine at 0.004 *M*, stimulate feeding in *L. decemlineata* larvae [Thorsteinson *et al.* (70)].

The chemotactic influence of minerals.—There has been some interest in the effects of mineral fertilizers supplied to the food plants on the nutrition of phytophagous insects. Observations of this kind have an ecological interpretation but do not provide critical data on the behavioural aspects of feeding.

The gustatory response of insects to NaCl and other minerals has been reviewed by Dethier (17). In general, reports of insect responses to minerals have been negative or indifferent, and results are usually summarized in terms of rejection thresholds.

Nevertheless, early baits for grasshoppers included salt as an ingredient intended to enhance palatability. The rationale of this procedure is said to be derived from the observation that grasshoppers gnaw pitch-fork handles, presumably stimulated by the salts derived from human perspiration. When it was found that salt adds little to the effectiveness of baits containing bran, the mineral supplement was eliminated.

Recent experiments on *Camnula* in our laboratory confirm the inhibitory effect of KCl on feeding responses to sucrose. A very different result was obtained when we studied feeding responses to potassium acid phosphate (KH_2PO_4). Like KCl, this mineral does not by itself evoke feeding responses and higher concentrations inhibit responses to sucrose. However, at 0.004 *M* concentration, KH_2PO_4 appreciably enhances the responses to 0.02 *M* sucrose [Thorsteinson *et al.* (70)]. Once again, we observe interacting effects of simple mixtures of chemical stimuli.

Undoubtedly these additive and synergistic effects are significant for an understanding of plant susceptibility to feeding injury by insects. Of course, the presence or absence of repellents and inhibitors are also highly relevant in this regard, but these are considered in a later section. It is interesting in a teleological sense that phosphates are more palatable than

chlorides inasmuch as phosphate may be considered more significant metabolically and is also more likely to be deficient in plants.

The chemotactic influence of organic acids.—Verschaffelt (73) reported that oxalic acid stimulates feeding by *Gastroidea viridula* (De Geer) larvae. Citric acid was added to some early grasshopper baits in Manitoba. Oxaloacetic acid and citric acid stimulate feeding in *Camnula* to some extent [Thorsteinson *et al.* (70)].

The chemotactic influence of vitamins.—The vitamins are a chemically heterogeneous group but may be considered together for convenience, inasmuch as their chemotactic significance has hardly been investigated. Certain water-soluble vitamins (ascorbic acid and thiamine) elicit a feeding reaction in *Chorthippus* [Thorsteinson (68)]. Thiamine and α -tocopherol are attractive to *Tribolium confusum* Duval [Losciavo & Thorsteinson (46)]. Considering the almost negligible attention devoted in this direction, these findings seem to foreshadow the existence of similar, as yet undiscovered, effects, but it is premature to attach decisive significance to them. However, the few data at hand are in harmony with the concept that many of the substances which chemotactically influence feeding behaviour are nutritionally important to insects.

The chemotactic influence of plant pigments.—There seem to be no confirmed reports of gustatory effects of fat-soluble pigments such as chlorophylls, carotenes, and flavones. The same appears true of water-soluble pigments. Many plants are rich in flavone glycosides. Chauvin (7) obtained a preparation containing flavone glycosides from potato leaves which stimulated feeding in *Leptinotarsa* larvae. However, flavones isolated chromatographically from potato-leaf extracts in our laboratory proved chemotactically inert for *Leptinotarsa* larvae [Thorsteinson *et al.* (70)]. On the other hand, chromatographic fractions containing sugars and amino acids or pure solutions of sucrose or γ -aminobutyric acid evoke considerable feeding in this insect. Presumably, Chauvin's preparation and also the extracts tested by Raucourt & Trouvelot (59) contained sugars and amino acids which are often difficult to separate from other substances in plant extracts. Our *Leptinotarsa* experiments constrain us to accept the hypothesis that oligophagy in this insect, at least, may be determined by "host avoidance" [Wardle (75)], rather than "host selection." A similar conclusion in regard to the food-plant selection by the adult of this same insect was reached by Jermy (36) by inference from the results of experiments of the "leaf sandwich" type introduced by Dethier. (11).

The chemotactic influence of glycosides.—The influence of mustard-oil glycosides on feeding behaviour and host selection of *Pieris rapae* (Linnaeus) larvae was the classic discovery of Verschaffelt (73). The same phenomenon was investigated more extensively for *Plutella* and reinterpreted by Thorsteinson (66). A number of insects are associated with plants that contain well known glycosides such as amygdalin, salicin, etc., but there

is as yet no evidence that these substances affect the feeding behaviour of the insects concerned. Such effects, if they exist, might elude discovery if investigators neglect to regard them as "synergizers" of response to more commonplace plant constituents (sapid nutrients). The possibility of such neglect is suggested by the fact that sinigrin does not by itself elicit much feeding in *Plutella*, nor does sucrose, for example, but in combination sucrose and sinigrin are effective feeding stimulants and the addition of other sapid substances further increases the response. Tentatively at least, we may interpret the influence of sinigrin and similar substances as a lowering of the thresholds of feeding response in certain phytophagous insects for sapid nutrients in the food plants.

The chemotactic influence of alkaloids.—There is no record that an alkaloid stimulates feeding by insects, but Kennedy & Stroyan (41) mention the unpublished finding of Smith (62) that sparteine seems to stabilize colonization of the aphid *Acyrtosiphon spartii* (Koch) on its food plants. The possibility that solanin might account for host selection in *Leptinotarsa* larvae was eliminated early by Raucourt & Trouvelot (59). Alkaloids are generally thought of as toxic substances and presumably insecticidal. Nicotine is certainly a striking example, having been commercialized as such for decades. The extensive studies of the alkaloids in Solanaceae by Schreiber (60), Buhr, Toball & Schreiber (5) and other German workers in relation to the beetle, *Leptinotarsa*, reveal that while some of these alkaloids may be toxic, the operative effect of tomatin and several others is chemotactic inhibition of feeding activity. On the other hand, nicotine and other alkaloids kill the larvae soon after ingestion before any inhibitory influence on feeding manifests itself. The inhibitory influence of demissin is somewhat delayed, as is graphically illustrated by de Wilde (80), but here the alkaloid seems to be relatively less toxic and death can be explained by starvation. These studies clearly indicate that many of the solanaceous plants containing toxins and feeding inhibitors also contain feeding stimulants for *Leptinotarsa*. The resulting implications for a general theory of food-plant selection is discussed in a later section.

The chemotactic influence of water in gustation.—It is rather difficult to evaluate water as a gustatory stimulant inasmuch as we tend to assume that taste substances must operate in solution for which the moisture is supplied either by the substrate or by the insect itself as saliva. However, since insects can be "thirsty," that is they will drink pure water, we must consider it highly probable that water provides some sensory stimulus to account for the nervous input required to evoke a drinking response. Wolbarsht (82) has reported water taste for *Phormia regina* (Meigen), and presumably similar perceptions remain to be discovered in other insects. Water is apparently not an "essential" feeding stimulus in the strict chemotactic sense for all leaf-eating insects since dry poison baits seem to be effective in the control of some insect pests, and in our studies of gustation of *C. pellucida*, cited previously, chemicals were tested satisfactorily in dry substrates. Chapman

(6) has correlated the food-plant preferences of the red locust, *Nomadacris septemfasciata* (Serville), with moisture contents, but the chemical constitution of the plants was not investigated and may well have provided alternative correlations.

GENETIC VARIABILITY IN FOOD-PLANT SELECTION

The innate variability among individuals within food-plant species is significant both to an understanding of food-plant selection by insects and insect resistance in plants, as was early recognized by Painter (54). The variability in food-plant preferences within an insect species has been clearly recognized by Dethier (18) who relates earlier reports to their importance in evolutionary theory and practical implications. This same variability presents a dilemma to the experimental investigator of food-plant selection who is confronted with the awkward choice of accepting high standard errors as measures of treatment differences or the loss of information concerning genetic segregation which is so important to the ethological and ecological interpretation of the data. At the risk of shocking those who worship "z" and "p" values, I venture to suggest that the second hazard is more serious for anyone especially interested in ecology, and in this perspective, at the present stage of knowledge, a qualitatively extensive, albeit only semi-quantitative, approach is the more fruitful alternative.

Some methods for studying food-plant selection by insects have been offered by Maltais (47), Nuorteva (53), and Thorsteinson (67).

PATTERNS OF HOST PLANT SELECTION

Phytophagous insects have been classified in various ways according to their food-plant preferences. These are briefly considered in the following paragraphs.

The economic entomologist groups insects according to agronomic criteria, recognizing such classes as vegetable, fruit, forage, forest, cereal crop insects, etc., or according to the part of the plant injured, e.g. foliage feeders, bark or stem borers, and root infesters. This classification obviously serves a utilitarian purpose but does not lend itself advantageously to analytical investigation of feeding habits.

A classification on a phytogeographical basis has been suggested by Hering (31). Hering coins the terms "xenophobie" and "xenophilie" which refer, respectively, to rejection and acceptance of plants indigenous to a region other than the aboriginal home of the insect. These terms may be useful in general discussions of quarantine problems, biological control of weeds by insects, zoogeography, and evolution. Their validity in relation to food-plant selection theory is rather limited because they skirt the fundamental, largely chemotactic basis of food-plant preferences. They do however imply the important fact that the food-plant range of an insect is potentially greater than regional records would indicate.

The classification accorded the most general recognition is implied by

the terms "monophagy, oligophagy, and polyphagy." Etymologically, these terms imply a numerical basis—referring to the number of plant species accepted as food by the insect species concerned. It does not seem to be quite clear whether the food plants should be enumerated by species, genera, or families. Certainly, an insect specific to a family such as the Cruciferae has a rather extensive food-plant range in terms of number of plant species. The most elaborate classification of this sort is the interesting memoir of Jolivet (37).

Dethier (15) has suggested that oligophagy would be best defined in terms of attractance of several different chemicals, as in *Papilio ajax* larvae. Attractance to one chemical, or to a group of chemicals confused by the insect as one, would constitute monophagy, and an insect specific to the large group of cruciferous plants and other plant families that contain mustard-oil glucosides, would be regarded as monophagous. However, *Plutella* larvae, whose food-plant range corresponds with the botanical distribution of mustard-oil glucosides [Thorsteinson (66)], will under laboratory conditions eat the leaves of certain legumes that do not contain these substances [Gupta & Thorsteinson (29)]. As discussed more fully above, the oligophagous habit in *Leptinotarsa* larvae may not be based on a restricted distribution of feeding stimulants, as has been assumed, but on the absence of feeding inhibitors in the food plants and the presence of inhibitors in all other plants. It is evident that the oligophagous food habit involves a variety of mechanisms which, when more fully understood, will not comfortably fall into a single class.

In the first attempt to relate polyphagy to chemotactic factors, all insects that feed on any plant free of feeding inhibitors were considered polyphagous [Dethier (15)]. While this is certainly true, it does not follow that polyphagous insects require no feeding stimulants or attractants. Furthermore, it is important to recognize that inhibitors and repellents are fully as significant to food-plant selection in oligophagous insects as they are in polyphagous insects [Buhr, Toball & Schreiber (5); Thorsteinson (68)]. It appears now that the Colorado potato beetle, *Leptinotarsa decemlineata*, an oligophagous insect, selects its food plants by much the same mechanism as do polyphagous insects [Thorsteinson *et al.* (70); Jermy (36)]. The investigations of Watanabe (76) and Ito (34) suggest to this reviewer that the silkworm, *B. mori*, a nearly monophagous insect, also utilizes similar host-selection mechanisms. Ito (33) has discussed food-plant selection in *B. mori*. Ito, Horie & Fraenkel (35) have demonstrated that maxillectomized silkworm larvae will feed for some time on otherwise rejected cabbage and cherry leaves—an indication of the significance of inhibition in the feeding behaviour of this insect—confirming the earlier work of Torii & Morii (71).

Painter (54) has pointed out that the concepts of oligophagy, and so forth, are incompatible with the phenomenon of insect resistance in individuals and varieties of a given food-plant species. This argument does not apply in this context to resistance of the antibiosis and morphological types

since insect behaviour is not involved, but resistance of the nonpreference type is clearly an awkward fact to fit into a scheme of classification of food-plant preferences predicated on botanical units at the species level not to mention genera and families.

If we are to retain terms like oligophagy, etc., it is desirable to attempt to define them more objectively. Possibly our difficulties have resulted from the priority assigned to botanical taxonomic arrangements; this is understandable considering it is so much easier to identify plant species than the chemicals they contain, not to mention the relative dearth of knowledge as to how these substances regulate feeding behaviour. It is, in retrospect, not to be wondered that difficulties are encountered when one attempts to fit our growing knowledge of chemotactic influences into imperfectly correlated, preconceived categories. If one recognizes that chemical stimuli are the predominating regulators of food-plant selection, then a classification of food preferences in purely chemotactic terms should be attempted. Afterwards, one may recognize whatever botanical correlations might emerge.

This approach requires that plants are thought of in terms of an assemblage of chemicals which provide chemotactic signal patterns perceptually significant for phytophagous insects. This abstraction is no less real than the morphological patterns that provide plant taxonomists with their basis for identification.

The probability of occurrence among plants of effective signal patterns, which excite feeding activity within the time-space environment of a given insect, determines the number of its potential food plants. An insect that is responsive to signal patterns of high statistical frequency among plants is polyphagous while insects in rapport only with signal patterns of lower frequency are oligophagous. In the case of a given insect species, the botanical distribution of effective signal patterns may be highly correlated with certain taxonomic groupings (for example, signal patterns that include a mustard-oil glucoside can be supplied by only a few plant families). The frequency of effective signal patterns in such cases is obviously determined by the number of species in that plant group. Since these plant groups might be small or large, the associated insects may be, in the literal etymological sense, either oligophagous or polyphagous. At this point, the following classification, of food plant preferences of phytophagous insects is offered, without benefit of a convenient nomenclature, as a basis for development as our knowledge increases.

CHEMOTACTIC CLASSIFICATION OF FOOD PLANT PREFERENCES

Type I—Feeding and oviposition are induced by chemical stimuli present in virtually all plants, but not all plants are consumed because some contain substances that inhibit the response to the feeding stimulants.

Subtype IA.—Feeding is inhibited by substances distributed more or less at random among plant groups, e.g. various grasshoppers and cutworms.

Subtype IB.—Feeding and oviposition are highly sensitive to inhibition by one or more substances in all plants except some of the members of certain taxonomic groups, e.g. *Leptinotarsa decemlineata* and possibly *Bombyx mori*.

Type II.—Feeding and oviposition are induced by chemical stimuli, one or more of which are extraordinary and occur in some plants but not in others.

Subtype IIA.—The botanical distribution of the extraordinary stimulus substances is highly correlated with natural plant taxonomic groups, e.g. *Pieris rapae*, *Pieris brassicae* (Linnaeus), and *Plutella maculipennis*.

Subtype IIB.—The extraordinary stimulus substances are sporadically or randomly distributed among plants. (This category is for the present hypothetical inasmuch as no appropriate examples come to mind.)

It requires notice that food-plant preferences must in many insects be determined harmoniously in both the larval (feeding) and adult (feeding, or oviposition, or both) stages. The type of food-plant selection mechanism in both stages may conform in principle to the same type or, conceivably, two different types.

REVIEW OF ANTECEDENT THEORIES

It is adequately established that the feeding behaviour of phytophagous insects and, therefore, food-plant selection is regulated for the most part by taste and smell. There seem to be two schools of thought concerning what plant constituents provide the stimuli that determine food-plant preferences. In several papers [Fraenkel (21, 23, 14); Lipke & Fraenkel (45)], the thesis is pronounced that food-plant selection is determined solely by the "odd" or secondary chemicals (e.g. essential oils, glycosides, alkaloids, etc.), which plants synthesize for no apparent metabolic purpose. A similar view is advanced, less categorically, by Dethier (18). There is, in fact, little doubt that many such substances do act as repellents or inhibitors of oviposition and feeding, and this fact alone would establish them as very significant determinants of food-plant selection.

A very few of these substances, as cited elsewhere, have been shown to function in reverse, stimulating oviposition, aiding in finding food, and stimulating feeding by insects specific to the food plants that contain them. Although a number of attempts to extend the list of such examples has proved unfruitful or indecisive, Fraenkel (24) and Lipke & Fraenkel (45) have stoutly argued that secondary chemicals are solely responsible for guiding phytophagous insects in general to their preferred food plants and providing the chemical stimuli required to induce feeding. Dethier (18) states that the causal role of nutrients in food plant selection is an open question.

Consider the statement of Lipke & Fraenkel (45, p. 32):

The most fundamental aspect of host selection in leaf eating insects inquires whether the selection is governed (a) by the nutritional superiority of the plant or region of the plant serving as food for the insect, or (b) by the presence or absence of attractants and repellents in plants of more or less uniform food value to which the parasitic species has become adapted. The latter view implies that the token stimuli, i.e. the substances which are responsible for the acceptance or rejection of the host, have no nutritional value for the insect in the sense that they are metabolized and incorporated into the host tissue or biochemically involved in tissue synthesis.

An examination of this quotation raises several questions about the validity and appropriateness of these propositions, including the following: (A) Is the "nutritional superiority" of the preferred food plants supposed to regulate (if it does so) the selection of food of insects through behavioural mechanisms? Certainly, any other kind of mechanism could operate only through a process of natural selection and is not relevant in the present context. If then, the mechanism has a behavioural basis, we must agree that it is improbable that insects can recognize the wholesomeness of a leaf—nutritiousness as such can scarcely constitute a feeding stimulus. (B) But, is there any reason why some of the nutrient substances in leaves cannot supply chemotactic stimuli that elicit feeding by insects not repelled by any of the other constituents of the leaves? (C) In proposition (b), why does the postulate that leaves contain attractants and repellents imply that all these substances are token stimuli of no metabolic use to the insects? [This is a restatement of point (B) which, however, related to proposition (a).] This summary exclusion of nutrients as potential feeding stimulants and regulators of food-plant selection is surprising in view of the fact that one of the most common nutritious constituents of plants (sucrose) has been known for many years to influence gustation in various leaf-eating insects [Dethier (11, 12)].

Even in insects that do not seem to respond to pure sugars, food-selection behaviour may be influenced by gustatory summation effects of saccharides with other substances [Eger (20); Thorsteinson *et al.* (70)]. That insects do, by and large, select food plants which are nutritious is attested by their successful survival, but it is not at all necessary that they sense the taste or smell of every essential nutrient. Sucrose, for example, occurs in nature in substrates (leaves or other plant tissues) that, generally speaking, also contain all of the other nutrients required by insects. Oddly enough, it is this same relative uniformity in leaf constitution that led Fraenkel (21, 24) to infer that it must be only "odd" chemicals that regulate food selection. Contributing to this conclusion is the apparent impression that nutrients occur in plants only as proteins, complex carbohydrates, and fats which, in the pure state, are presumed to have no distinctive taste or smell [Fraenkel (24)]. It is, however, generally known that simple carbohydrates (sugars), amino acids and amides, organic acids, and various other metabolic products

occur in plants in the free form. Because many of these substances initiate and sustain feeding in insects as mentioned in previous sections, they have been designated as "sapid" nutrients and their role as feeding stimulants has been discussed by Thorsteinson (68, 69). Of course, not all of the nutrients essential to insects occur in free form in plants, nor are those that do necessarily essential in the metabolic sense. However, it is quite clear that some of the nutrients in plants act as gustatory indicators of a suitable food substrate. From this point of view, there is little reason why we should not include such nutrients with secondary substances in the class of token stimulants.

There remains the fact that some secondary substances (mustard-oil glucosides) are essential to the food-selection behaviour of at least three insect species [Thorsteinson (66); Verschaefelt (73)], although even here, the sapid nutrients are equally essential to food selection. It would be tempting to generalize from this that secondary substances are necessary feeding stimulants at least for insects highly selective in their food-plant preferences. The Colorado potato beetle, *L. decemlineata*, is highly selective, yet the evidence indicates that no secondary substance is involved as a feeding stimulant or attractant. We can alternatively explain its food preferences in terms of avoidance of feeding inhibitors in plants outside its food-plant range [Jermy, (36)]. Removal of the palpi results in acceptance of leaves refused by the intact larva, an indication of the significance of inhibitors [de Wilde (80); Chin (9)]. Which of these two mechanisms will explain the majority of food-plant specificities among oligophagous insects can be established only after a great many more cases have been successfully investigated.

Discoveries of new dimensions in food-plant selection, not predictable at the present time, may be expected. It can already be foreseen that various permutations are possible when one considers that oviposition behaviour as well as feeding-response mechanisms, contribute to the overall regulation of food-plant selection in many insect species. In aphids, food-selection patterns appear to be somewhat more complex, considering the phenomenon of host-plant alternation that occurs in this group. This circumstance prompted Kennedy (38) to propose the "dual discrimination" hypothesis. Since the same species of aphid is sensitive at one period to differences in leaf age or quality and at another time to host-plant species, there is an apparent duality of behaviour, but this is hardly less puzzling than the neurophysiological reversal of behaviour mechanisms relating to feeding and dispersal that Kennedy (39) himself later elucidated. Although secondary chemicals may be involved in recognition of food-plant species and possibly, as Lipke & Fraenkel (45) argue, even in selection of leaves, it is probable that nutrients contribute significantly to the regulation of both of these food-selection patterns.

In a very recent paper, Frankel (24) has suggested that plant parasites, especially insects, constitute an evolutionary cause for the development of odd chemicals in plants. This, however, is not compatible with his other

claim that secondary substances are the sole attractants in plants that regulate food-plant selection. It is essential to realize that "selection" of a food plant is equivalent to accepting it, and real acceptance cannot occur in the absence of some kind of chemotactic feeding stimulus. If insects "caused" plants to develop secondary chemicals, the plants must have been subject to insect attack before and, therefore, must have contained feeding stimulants from the beginning. Since these stimulants could not (by this theory) have been secondary chemicals they must have been nutrients. If nutrients regulated feeding behaviour before plants developed secondary chemicals, it is hardly conceivable that they do not significantly influence feeding behaviour and, hence, food-plant selection today.

A REVIVED CATENARY THEORY OF HOST-PLANT SELECTION

Dethier (18) has summed up the whole problem of food-plant selection in two questions: "(1) how is the preference implemented? (2) what is the genetic basis and evolutionary history of specific plant preferences?" Regarding the second question he (18) has applied his extensive erudition with a success quite beyond my capacity to emulate, much less excel. Perhaps, one should, however, question the concept of "passive selection" on the grounds that all selection is passive in that inhibitors and repellents exercise a veto in the food-plant selection of all insects, and, on the other hand, all food selection is active in that the insect responds to effective feeding and oviposition stimulants when they encounter them. The evolutionary acquisition of responsiveness to new stimuli, if obligatory, does not make the selection more active (it is at least as likely to restrict it); although, by way of compensation, the new response, if olfactory, may aid the insect in finding the food plants within its reduced botanical range. The exclusion of nutritional requirements as a causative evolutionary factor is also open to question in the light of the later findings of Beck (3) and others.

Turning to the first primary question of how the preference is implemented, the two precepts advanced by Dethier (18) can be restated as follows: (A) Although physical stimuli define the environment for oviposition and feeding, the decisive act of host selection is regulated predominantly by the chemical sense. (This has been discussed at length in an earlier section.) (B) The behaviour of phytophagous insects toward their food plants is best understood when analyzed into a series of component phases. Dethier (18) lists three such components: (a) orientation to the food, (b) biting response, and (c) continued feeding. This series becomes more complete and acquires a regenerating path if we add (d) dispersal. The necessity of regarding dispersal as reciprocally antagonistic to the first three components in the cycle has already been discussed.

It is now required to recall the older concept of stimulus—response chains, reviewed, for example, by Wigglesworth (78) and more recently by Nuorteva (52). In the light of more recent studies, however, we must recognize not only the principle that a given link (reflex arc) in the chain facilitates the

manifestation of the following link but also that each unit response is likely to require the simultaneous influence of more than one eliciting stimulus. This latter principle applies not only to the interaction of two different types of stimuli, e.g. color and odor, but also to summation effects of several stimuli of the same general type, e.g. chemotactic (say, sinigrin and sapid nutrients). The links in the chain of stimuli are, therefore, to be regarded as occurring in parallel as well as in sequence. Recognition of this principle will help to fend off the human temptation to exaggerate the significance of an individual, isolated stimulus, however intriguing it may appear.

It is not stretching the simile too far to regard these circular chains as larger links of greater branching chains in an evolutionary system. The whole problem of food-plant selection, certainly in the evolutionary perspective and even on the relatively simpler contemporary scale, is best understood in terms of a concatenation of unit effects. While these unit phenomena require to be studied one or two at a time, their significance can only be fully appraised in the aggregate.

RECAPITULATION

In an attempt to provide a fresh basis for the development of our understanding of food-plant selection, the following symbolic statement, modified from Thorsteinson (69), is offered:

$$F \rightleftharpoons -I - D + E_{an}(E_p)$$

An optimal feeding response, F , implies that the substrate is devoid of feeding inhibitors, I , or deterrents, D , and contains the chemotactic stimulants, E , essential to elicit feeding. The essential feeding stimulants of general botanical distribution, to the best of our present knowledge, are sapid nutrients, E_{an} . In order to encompass in this general statement the behaviour of any insect species that require special feeding stimulants of limited botanical distribution and presumably of no strictly metabolic consequence, we include the removable term (E_p). This represents, in anthropocentric terms, a "piquant" stimulus; the objective rationalization of this designation follows.

In the only definitive, established illustrations of essential feeding stimuli of limited botanical distribution, where the substances have been identified [Verschaffelt (73); Thorsteinson (66)], neither the nutrients (in this case only potentially sapid) nor the metabolically neutral, piquant stimuli are sufficient independently to elicit an optimal feeding response. In view of this and of the fact that piquant substances are not essential to feeding activity and food-plant selection in many insects, including some with a very restricted food plant range, it seems appropriate to regard these special stimuli as agents that prime receptivity to sapid nutrients (lower response thresholds thereto) much as light perceived by the dorsal ocelli is said to prime receptivity in other nervous circuits [Wigglesworth (78)].

This account is applicable with no essential modification to the adult as well as the larvae in species that depend on the same food in both feeding stages, although the stimuli required to elicit oviposition or even feeding in the adult may not coincide precisely with those related to feeding by the larvae. In Lepidoptera and other insects in which the adult female either feeds on quite different plants or perhaps does not feed at all, there does not seem to be any question of nutrients (directly important to the adult) influencing her selection of food plants for her offspring [Dethier (18)]. However, this does not altogether exclude the possibility that oviposition may be influenced by chemotactic stimuli derived from substances of some nutritional use to the larvae. Dethier (15) mentions an early report by Hancock of a grasshopper that briefly tastes the plant before ovipositing on it. Perhaps the ovipositor of some phytophagous insects may be equipped to perceive chemotactic stimuli as has been reported for hymenopterous parasites by Dethier (14).

INSECT RESISTANCE IN PLANTS

Food-plant selection, in the sense that it is equivalent to food-plant acceptance, is related to susceptibility of plants to insects. On the other hand, the feeding behaviour of insects could not simulate a "selection" characteristic unless some plants are rejected. This platitude relates food-plant selection to insect resistance in plants.

In the context of food-plant selection behaviour only the "nonpreference" type of resistance is of concern, not the other two types, "antibiosis" and "tolerance," recognized by Painter (55). Antibiosis, however, must play some indirect, evolutionary role in food-plant selection. The class of plants acceptable to insects is the sum of the classes susceptible and tolerant; these two groups are distinguished only for practical purposes and do not represent different basic behavioral mechanisms in insects. It is of special interest that the possibilities are as great for finding plant resistance to polyphagous as to oligophagous insects [Painter (55)]; this seems to be related to the fact that the chemotactic sense seems to be as highly developed in one group as the other [Thorsteinson (68)].

Morphological resistance is rarely, if ever, independent of other types of resistance, and it is to be noted that Painter (55) does not recognize morphological resistance as one of the primary mechanisms. Phenological resistance is probably of considerable significance in nature, but it is a consequence of dissynchronization of growth patterns of insects and plants and, from the point of view of feeding behaviour, is only accidental.

The experiences of investigators of insect resistance in plants is a rich source of inspiration for the experimental study of feeding behavior in phytophagous insects (55, 56). It is gratifying to observe that the more critical experimental studies of feeding behavior are shedding light on insect resistance and susceptibility in plants. There is perhaps no field of experimental investigation of phytophagous insects in which the discovery of new fundamental

biological relationships and potentially useful findings overlap to a greater degree.

CONCLUSION

The extraordinary variety of insect-plant relationships is likely to be based on more diverse mechanisms than can be foreseen at present. The promise of rewards is rich for investigators who approach the problem free of deeply rooted bias, and they will be able to improve on this account before long.

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SOME RECENT CONTRIBUTIONS TO THE STUDY OF THE DISTRIBUTION AND ABUNDANCE OF INSECTS¹

BY H. G. ANDREWARTHA

Zoology Department, University of Adelaide, Australia

AND

L. C. BIRCH

Zoology Department, University of Sydney, Australia

According to the theory of environment that was developed by Andrewartha & Birch (4), the environment of any animal comprises four components—weather, food, other organisms (of the same or of different species), and a place in which to live. Theoretically, an animal's chance to survive and multiply might be said to depend on its whole environment, but in practice one or several components usually turn out to be important enough to account for nearly all the variability that has been observed in the density of the population (or populations).

In putting forward this theory we rejected the traditional subdivision of environment into physical and biotic factors and "density-dependent" and "density-independent" factors on the grounds that these were neither a precise nor a useful framework within which to discuss problems of population ecology. It seemed to us that there was a sense (though not a particularly important one) in which all components of environment could be considered as "density-dependent," if it were meant that birth rate and death rate were functions of density. This was a broad definition of "density-dependence." It certainly would not be valid if we had used Nicholson's (39, 40) definition of "density-dependent" factors as those whose intensity (e.g., the numbers of an obligate predator) increases with increasing density of the population. This difference in definitions of density-dependent factors seems to have caused some confusion among certain readers. But this is just part of the confusion which inevitably results from the use of these terms, which mean different things to different people. Andrewartha (1) drew attention to a variety of meanings attached to the term "density-dependent" factor. Discussions between Solomon (47) and Varley (49) have further emphasized the ambiguities and have convinced us, if we needed further convincing, that this particular subdivision of components of environment cannot profitably be used to explain the densities of natural populations [see also Andrewartha (2, 3)]. We have therefore avoided these terms and the polemics that are associated with them.

Instead, we have summarized the results of a number of investigations that are important for their contribution to the general theory of ecology

¹ The survey of literature pertaining to this review was concluded in April, 1959.

and that show how the abundance of natural populations of insects may be determined by weather, food, other organisms, and a place in which to live. Not all the variability that may be observed in the densities of populations may be explained in terms of environment because some of it may be associated with genetical variability. So we have included some recent studies on races of insects.

WEATHER

A general account of the role of weather in determining the numbers of animals has been given by Andrewartha & Birch (4) and Birch (7).

DISTRIBUTION AND ABUNDANCE OF *ONCOPERA FASCICULATA*

The ecology of the hepialid, *Oncopera fasciculata* (Walker) in South Australia was studied by Madge (30 to 35). This lepidopteran is indigenous to southern Australia. It occurs as a pest of pastures mostly comprised of the annual subterranean clover and a variety of annual grasses or, in moister places, perennial clovers and grasses.

The climate in the area is broadly like that around the Mediterranean Sea: i.e., most of the rain falls during the winter, which is mild; the summer is hot and dry. The annual species of clovers and grasses produce seeds which lie dormant during the summer and germinate with the "break" of the rainy season in the autumn. The life cycle of *O. fasciculata* is attuned to this climate. The moths lay their eggs in the spring after the grass has grown tall, but before it has begun to wilt. The eggs hatch in about three weeks. At first the caterpillars live on the surface of the ground, but within a week or so they make vertical burrows in the soil where they live during the day, emerging at night to feed. They pupate during spring and emerge as moths soon afterward.

Madge studied the various stages in the life cycle in order to discover the likely causes of death at each stage. The adults usually mate and lay their eggs within a few days of emerging, and it seems likely that most of them live long enough to lay most of their eggs. A female may lay up to 2000 eggs. They choose areas where the pasture is long and dense. The eggs are placed on the surface of the ground usually in heavy concentrations because a moth is likely to flutter about on the ground laying most of her eggs within a few yards of the place where she landed after her nuptial flight.

The eggs, being on damp soil under dense vegetation are well protected from desiccation and it is probable that most of them hatch. But while the young larvae are on the surface of the soil or are still near the surface in shallow burrows, the soil is usually beginning to dry out. Madge measured the survival rate of young larvae in a series of square-yard plots. There was a significantly higher survival rate on plots under dense shelter than on those that had been thinned or mown. The survival rate was the same on the plots that started with 50 and 1000 eggs; i.e., the initial density had no influence on the survival rate. This relationship between the amount of shelter provided by the vegetation and the hazards of desiccation is especially

interesting in view of the observation that the moths seek dense high vegetation under which to lay their eggs. Notwithstanding this adaptation, the death rate of the young larvae at this stage may be high, up to 100 per cent in all but the most favourable places when the spring is dry. Madge showed that the death rate might well be high enough to cause a rapid decline in density of the population when more than half the days between September 1 and November 30 were rainless. On the other hand, if rain fell on many more than half the days during this period, the population might increase rapidly.

From an analysis of the meteorological records for 94 years Madge showed that, on the basis of rainfall, a "favourable" spring might be expected to occur about once in eight years. But since at least two such years in succession are needed to produce a plague of *Oncopera*, it appears that plagues might be expected about once in 77 years. Of course minor outbreaks might be expected more frequently.

The most serious outbreak of *Oncopera* that is known occurred from 1948 to 1950. Some larvae died from starvation, especially in the central parts of large "patches" where the crowding was intense. But even during the height of the outbreak there were still many thousands of acres of rich pasture where there were few or no *Oncopera*. The outbreak came to an end with a change in the weather before there was any shortage of food for *Oncopera* over the area as a whole. This insect's usual condition between outbreaks is that of a rare species surrounded by an abundance of food.

Outbreaks occur less frequently and the caterpillars are less abundant on meadow podsols than on terra rossa and volcanic soils. Because these soils are moister during the spring, the hazards from desiccation at this stage may be less. The other extensive soil type of this region, that of the sandy ridges, supports no *Oncopera*. The sand has poor capacity for water, and it supports only a sparse vegetation which provides inadequate cover.

Virtually all variability in the numbers of *Oncopera*, in space and in time, may be explained in terms of the interaction between weather and a place in which to live. The emphasis may be placed on one or other of these components of environment, depending on which aspect of the variability has to be explained. Outbreaks are likely to occur on the well-developed farmlands when the weather is favourable. But, no matter how favourable the weather may be, *Oncopera* are likely to remain scarce or absent from the sandy ridges and from any undeveloped areas that still carry the native vegetation, because such areas provide very few places suitable for *Oncopera* to live in. Similarly, the big increase in the abundance of *Oncopera* in the area as a whole that has occurred during the past 40 years has been caused by the development of rich pastures on the farms and the consequent enlargement of the area suitable for *Oncopera* to live in.

ABUNDANCE OF *APHODIUS HOWITTI*

The larvae of the scarabaeid, *Aphodius howitti* Hope, occasionally do severe damage to pastures over a wide area of south-eastern Australia

[Carne (19); Maelzer (36)]. In the large area where its distribution overlaps that of *Oncopera*, *A. howitti* occurs on the same sorts of soils and it is, like *Oncopera*, largely restricted to well-developed pastures. In these circumstances one might expect to find that the two species are important components in each other's environment. But this is not so because the behaviour of the adults in seeking places to lay their eggs keeps the two species separate.

The life cycle of *Aphodius* occupies one year. From September to December the larvae are in diapause in closed earthen cells about six inches below the surface of the soil. They pupate about the end of December and moult to adults during January, which is the height of the hot, dry summer. The beetles remain in their cells until stimulated by a summer rainstorm. One or two days after rain they emerge and usually they fly some distance away before burrowing into the ground to lay their eggs. They have a strong preference for moist soil for oviposition and rarely if ever burrow into dry soil. The amount of rain that falls with a summer thunderstorm is often slight. A fall of 0.25 in. may be enough to stimulate the beetles to emerge; but such a small amount of rain falling during the summer is not likely to make much impression on the dry soil in an area dominated by deep-rooting perennial grasses, nor is this much rain likely to wet the soil under a dense cover of dry stubble. In places where the covering of stubble is sparse, however, the soil may absorb most of the rain and remain moist enough to attract *Aphodius*; it is in such places that *Aphodius* lay most of its eggs. When little rain falls during the egg-laying period the eggs tend to be concentrated in the places where the cover is most scanty; when rain is more plentiful the eggs are usually distributed more widely. But in either case, this behaviour causes *Aphodius* to avoid the densely covered areas in which most of the *Oncopera* are concentrated.

The bare or sparsely covered places where *Aphodius* lays its eggs may develop without the interference of any external agency in pastures that are dominated by subterranean clover or in which the weed *Cryptoslemma calendulaceum* is prevalent because these plants produce a thin, nonpersistent stubble. Or the grazing of many sheep may leave an area sparsely covered by only a thin stubble, and the same condition may be caused by the feeding, during the previous winter, of many larvae of *Aphodius* or *Oncopera*. Thus, in an area where the pastures are generally very dense the presence of *Oncopera* may ameliorate the environment for *Aphodius* by increasing the area suitable for oviposition. Conversely the feeding of *Aphodius* may make an area unsuitable for *Oncopera*, but as there are always many other areas that remain suitable even when *Aphodius* is more abundant, this does not make much difference to *Oncopera*. In practice one finds that the two species influence each other's chance to survive and multiply scarcely at all.

The areas that are suitable for *Aphodius* to lay eggs in may be measured in acres or in square inches. For example, in pastures of annual clover and grasses in which the nitrogen in the soil is building up to the level which just

permits the entry into the sward of *Cryptostemma calendulaceum*, these weeds may be scattered sparsely through the pasture. They grow in a flat rosette which crowds out the other plants during winter but dries to a thin insubstantial stubble during summer. The beetles may seek out even these small favourable areas while neglecting the rest of the paddock where the stubble is dense. This may lead to wide scattering of the population even in a year when the oviposition period was dry. But in general there is a tendency for the population to be more widely scattered when the egg-laying period is rainy and more patchy when it is dry. Thus the population tends to be more widely and evenly distributed when it is large and increasing (see below).

The larvae are present from February to December. At first they remain deep in the soil near where they have hatched. When the rainy season breaks with the first substantial autumn rains, they come to the surface and construct vertical burrows, open at the top, in which they live during the day; at night they emerge to forage for food. They are fully grown by about August, but they remain as larvae in diapause until December, when they pupate.

Maelzer (36), with a series of observations and experiments in the field backed by complementary experiments in the laboratory, was able to recognize three stages in the life cycle when the chance of surviving may be rather small. (a) In the absence of any effective fall of rain during January and February, the beetles may not be stimulated to leave their cells in the ground or they may emerge but die without laying eggs. (b) In the absence of rain during the autumn (March to May) the young larvae may not be stimulated to come to the surface and many may die from desiccation and starvation without moving far from the place where they hatched. For example, Maelzer estimated that about half the larvae in one area that he studied died from this cause in 1955 and that in 1945 the death rate from this cause probably approached 100 per cent in many places. (c) Excessive rain during winter (May to August) may favour the development of the fungus *Cordyceps* which may kill many larvae; and the larvae that are living on the poorly drained meadow podsols may in addition be drowned during wet weather. Apart from *Cordyceps* no parasite, predator, or disease of any importance was found.

Maelzer found that there had been at least three major outbreaks of *Aphodius* between 1927 and 1958. The prevailing low numbers between outbreaks, the build-up to an outbreak, and its subsequent decline could all be adequately explained by reference to the weather as it influenced the survival rate during these three critical stages of the life cycle. It seems that recurrent drought in the autumn offers the most serious check to the multiplication of *Aphodius* in South Australia.

Carne (19) studied *Aphodius* near Canberra and reached quite a different conclusion. He observed that "the abundance of the species fluctuates greatly from year to year." And he explained these fluctuations as follows: (a) The larvae that are not living in local concentrations (which

Carne called "crowded situations") may be ignored because they constitute a negligible part of the whole population. (b) The density of the population in a "crowded situation" is always the same because *Aphodius* "produces a large surplus of offspring and so tends to multiply," but a density-governing factor (larval combat) regulates mortality so that it "approximately counter-balances the surplus of offspring produced," and thus "the number of adults produced per unit area of the crowded zones is approximately constant. (c) It follows from these two points that the number of *Aphodius* in the whole population depends on the total area occupied by the "crowded situation." Carne did not say explicitly what determined the total area occupied by the "crowded situations," but he stated his general conclusion as follows: "These observations demonstrate the existence of density-induced mortality which can regulate populations at densities related to the prevailing conditions." Presumably the "prevailing conditions" determine the total area occupied by the "crowded situations," but the inference is not quite clear.

Maelzer (36) thought that Carne's conclusions were unrealistic, and he criticized them at length. The following criticisms are largely based on points made by Maelzer.

(a) Carne's assumption that the abundance of *Aphodius* could be explained by reference only to the events that occur in "crowded situations" is unrealistic because larvae that are scattered sparsely often constitute the major part of the population of any substantial area. Moreover, this proportion is likely to be larger when the population is large and increasing.

(b) A realistic account of the events in a "crowded situation" cannot be given in terms of Carne's density-governing factor alone because, as Maelzer showed, there are occasions when a large proportion (even up to 100 per cent in places) of the larvae in crowded, as in uncrowded, situations die from desiccation and starvation without coming to the surface.

(c) Carne's conclusions concerning what happens in the crowded situations are based on inadequate evidence. From the evidence presented in his paper there is no basis for supposing that "larval combat" is primarily responsible for the reduction in density of *Aphodius* in crowded situations. Larval combat may account for high mortality in some places when the crowded area is so large that the larvae cannot disperse without entering another crowded situation. No evidence is given to indicate how large such an area would have to be for combat to occur instead of larvae simply moving into a less crowded area of little or no combat. Carne and Maelzer both give evidence that larvae in crowded areas do disperse without combat. But in the theoretical parts of his paper (19), Carne seems to assume that the dispersal of larvae is negligible. For example, his general explanation of the density of the population (p. 304) implies that all the larvae occur in "crowded situations" and that all "crowded situations" are so uniformly crowded and so large that dispersal within them or around their periphery is negligible. In support of these assumptions Carne states (p. 297), "Dispersal of larvae from patches of high density was observed to be of the

order of three to six feet over the whole season, irrespective of the size of the patch." And on page 300 he says, "Observations showed that the rate of dispersal is too slow to prevent combat from occurring." But we are not given the evidence that would allow us to evaluate these general statements. They are, to say the least, not supported by the description of the behaviour of the larvae that Carne gave in the early part of his paper. For example, many patches are small (pp. 293, 294); a larva may crawl 10 to 14 in. in a minute (p. 282); and 80 per cent of larvae crawled out of an area eight feet square quickly enough to prevent any of them from being bitten (p. 300). A similar criticism applies to Carne's Figure 12 because we are not given the data from which it was constructed or told how this was collected. We are told (p. 297) that these are typical examples of population density curves and (p. 298) that they represent areas of one acre selected for their uniformity of infestation. Yet in the earlier part of his paper Carne showed that "uniformity of infestation" is atypical for this species. Much more evidence than is given by Carne would be necessary before one could legitimately conclude that larval combat was of importance in determining the numbers of *Aphodius*.

(d) Carne and Maelzer agreed that it is unusual to find more than about 20 larvae per square link ($=0.405$ sq.m.). Maelzer emphasized that densities much lower than this are often found and Carne's Table 5 (p. 289) shows densities from 0.1 to 27.5 per square link in July. But in seeking to explain his findings in terms of a "density-governing factor," Carne apparently overlooked the need to explain how densities much below 20 are often encountered in nature. This is typical of the difficulties that arise when the concept of density-dependent factors is applied to nature.

OTHER ANIMALS OF THE SAME KIND

Andrewartha & Birch (4) pointed out that an animal's chance to survive and multiply may be small when there are too few or too many of its own kind in its environment. The hazards associated with "underpopulation" may take a variety of forms: the risk of not finding a mate may be a fairly common one. A series of papers by Bushland & Hopkins (15, 16), Lindquist (29), Knipling (28), Baumhover *et al.* (6) and Bushland *et al.* (17) describe how this principle was exploited in order to exterminate a population of *Callitroga hominivorax* (Coquerel).

On Curaçao this screw-worm used to breed chiefly in wounds on goats and sheep. The number of *Callitroga* on the island when the exercise began was not known, but there was some evidence that there were fewer than 500 per square mile, probably many fewer. The area of the island is 170 square miles.

The purpose of the exercise was to exterminate *Callitroga hominivorax* from the island by setting free, amongst the natural population, large numbers of males that had been sterilized by exposure to γ -rays. The theory behind this method may be stated briefly. Provided that the sterile animals are likely to be as successful in mating as the normal ones, then the presence of a

large excess of sterile males in the area is likely to lead to the production of infertile eggs. If the number of sterile males in the area is maintained, by the continuous release of new ones, while the number of normal males is decreasing as the population decreases from generation to generation, there may come a time when the probability of a fertile mating becomes effectively zero, and the population might be expected to die out. In the final stages, when the population has become sparse, the chance that a female will meet a fertile male may be small, even in the absence of sterile males. But in the early stages, when the population is dense, the probability of a fertile mating may be high unless the sterile males greatly outnumber the fertile ones. The extermination of the population might be expected to be more certain and to be achieved more economically if (a) only males are released, (b) all of them are sterile and (c) none of them has a chance of regaining any fertility before it dies. But none of these conditions is absolutely essential for success.

The attempt to exterminate *C. hominivorax* on the island of Curaçao was strikingly successful. Part of its success may have resulted from qualities in *Callitroga* which made it especially susceptible to this method. The females usually mate only once, the males several times. For about six months sterilized males were released on the whole island at the rate of about 400 per square mile per week. The number of egg masses laid per week on goats exposed in 11 pens distributed over the island fell from 49 to 0 within a month or so and the percentage of infertile egg masses increased from 69 to 100 per cent in about the same period. Observations were continued until the authors concluded that there were probably no *C. hominivorax* left on the island. A similar campaign to eradicate *Callitroga* on the mainland of the United States is in progress at the present time.

Knipling (28) reviewed these results and discussed the possibility of using the same method with other species. Small isolated populations such as might occur when a pest has been recently introduced into a new area might be eradicated in this way. Or this method might supplement the use of parasites and insecticides for controlling pests that are firmly established. There are doubtless many species for which this method would prove impracticable, but when it is practicable this approach may well turn out to be economical and more effective than any other method. In Hawaii the influence of radiation on *Dacus dorsalis* Hendel has been studied with this end in view [Steiner & Christenson (48)], and in Australia similar work has been started on *Dacus tryoni* (Froggatt).

FOOD

Andrewartha & Birch (4) pointed out that it is useful to recognize two ways in which food may influence an animal's chance to survive and multiply. The food may be sparsely distributed relative to the animals' capacity to disperse and find it, or it may be concealed from the animals in some other way. In these circumstances there may be such a small proportion of the

food found and eaten that there is no likelihood that the total stock of food in the area will be depleted to any important degree by the feeding of the animals. Yet, at the same time, many animals may be dying or failing to reproduce through lack of food. We called this a "relative" shortage of food to distinguish it from circumstances in which the food is readily accessible to the animals and a large proportion of it is likely to be eaten when the population is dense. We called this an "absolute" shortage of food. We cited, as examples of the relative shortage of food, *Thrips imaginis* Bagnall, *Ixodes ricinus* (Linnaeus), and a number of other insects.

EXTERMINATION OF TSETSE FLIES

Potts & Jackson (42) described a striking example of how the principle of the relative shortage of food was exploited to exterminate a population of tsetse flies, *Glossina morsitans* Westwood, from an area of 600 square miles of bush in Africa. The tsetse fly sucks blood chiefly from the larger species of ungulates. It must feed frequently, especially during hot weather, but it does not stay on or near its host after taking a meal; so each meal is preceded by an independent search for a host. If hosts are few or sparsely distributed, a fly may have little chance of finding enough food to produce many or any offspring before it dies, despite the fact that the amount of blood in even one beast would be more than ample to support many flies. Jackson asserted that there must be a particular density of hosts at which the flies would be able to find food just often enough to maintain births equal to deaths and thus keep the population steady. Consequently, in order to exterminate the flies it should not be necessary to exterminate the hosts but merely to reduce the population below this particular critical density.

In accordance with this idea, 50 to 100 hunters were employed to shoot as many as possible of the larger mammals which are the favoured hosts of the tsetse fly. At the beginning of the experiment there were about 10 such animals to the square mile. During the next five years about 8000 animals were shot, including many that came in from the outside. By the end of five years the numbers of the more important species had been greatly reduced but a small herd of elephants and many small ungulates were still living in the area. Altogether the animals remaining at this time still contain in their bodies many times more than enough blood to provide food for all the tsetse flies that had been present at the beginning. Nevertheless the animals were few enough and they were distributed sparsely enough to give the flies only a poor chance of finding enough food. By the end of the five years Potts and Jackson were satisfied that *G. morsitans* and *Glossina swynnertoni* Austen had been exterminated and that *Glossina pallidipes* Austen had been either exterminated or reduced to extremely low numbers.

BIOLOGICAL CONTROL OF WEEDS

An "absolute" shortage of food implies, by definition, that a large proportion of the food in the area is eaten by the animals. In these circumstances

it often, though not invariably, happens that the amount of food in the area largely depends on the number of animals feeding on it. The ecology of the codling moth [*Carpocapsa pomonella* (Linnaeus)] provides an exception to this rule [Andrewartha (1)]. The biological control of weeds depends on this reciprocal relationship between the amount of food and the number of feeding animals [Andrewartha & Birch (4), Figures 11.04, 14.02, 14.07]. An early example of the biological control of a weed was the control of prickly pear, *Opuntia*, in Queensland by *Cactoblastis cactorum* (Berg). Huffaker (22) described a recent example which was also strikingly successful, namely the control of *Hypericum perforatum* (Linnaeus) in California by two species of Chrysomelidae, *Chrysolina quadrigemina* (Suffrian) (= *Chrysomela gemellata auctorum*) and *Chrysolina hyperici* (Forster).

Huffaker (22) also discussed a number of plants commonly regarded as rare species whose scarcity might reasonably be attributed to the feeding of indigenous insects. These examples indicate that the commonly observed scarcity of phytophagous animals relative to their stocks of food which has been commented on by Darwin, Elton, Andrewartha and Birch, and other naturalists is not a universal condition of all phytophagous animals. This, of course, has never been doubted, but Huffaker has suggested that the statements on this subject sometimes quoted from Darwin and other naturalists may be overstatements. In further support of this argument, Huffaker suggested that perhaps not enough weight has been attached to the possibility that only a certain part of a plant or a certain part in a certain condition may be suitable as food for a particular species of animal; and as a consequence, the stocks of food are often more scanty than would appear to a casual observer. This is an interesting idea which should be studied. But, in relation to general theory, it should be noted that confirmation of Huffaker's hypothesis, in any particular instance, would still leave the matter open as to whether the animal's chance to survive and multiply could best be explained in terms of the principle of a relative shortage of food or of an absolute shortage of food.

OTHER ORGANISMS OF DIFFERENT KINDS

NONPREDATORS

Although it is frequently asserted or assumed that the number of a particular species is affected by the presence in its environment of some other species which uses the same food or directly interferes with it [see Birch (8)], surprisingly few critical studies have been made. Brian's work on ants referred to below in an exception in this respect.

Ants.—Ants of one species or other influence the numbers of some other species of ant in their environment more commonly than is the case with other insects. It seems more usual with other insects that two or more species may be found using the same food or space without seriously affecting the numbers of each other. We refer to the studies of Ross on leafhoppers (43)

and of Sokoloff on *Drosophila* (44, 45) as examples of this. The so-called "Gause's Law" has been regarded by many as the appropriate approach to this aspect of animal numbers. If there were overlap in requirements for essential resources by two or more species, this was taken to mean that there was a superabundance of common resources, for it was argued that in the absence of such superabundance one species would inevitably replace the other. If there were no overlap it was argued that one species has displaced another through more successful acquisition of needed resources or through some form of direct interference. The argument is, to say the least, an oversimplification. A growing number of ecologists is becoming unwilling to accept the so-called law as a general principle of natural population. [See reviews by Andrewartha & Birch (4); Hutchinson (25, p. 419); Nicholson (40, p. 170); Solomon (46, p. 129).]

In the west of Scotland, *Myrmica rubra* (Linnaeus) and *M. scabrinodis* Nylander sometimes occur together and sometimes separately. In woodland only *rubra* is found; in heath heather and felled woodland both are found, but in these places *M. scabrinodis* lives in short turf and *M. rubra* in longer grass-herb vegetation. This segregation within the heather or felled woodland is evidently not brought about by selection of different places by the ants; it is caused, at least in part, by an active interference of one species by the other [Brian (11)]. Colonies are founded in summer. *M. rubra* are the first ants to arrive; their foundress queens form colonies in the warmest places which are wet but not waterlogged. *M. scabrinodis*, which arrives a little later, selects the same sorts of warm and well-drained sites for its colonies. Some or perhaps many of the suitable sites have already been colonized by *M. rubra*. The second arrivals, namely *M. scabrinodis*, lay siege to the nests of *M. rubra*, remove the appendages of the workers and devour their males. This, especially when associated with dry weather, is enough to cause *M. rubra* to evacuate its nests and *M. scabrinodis* takes over the drier parts of areas previously occupied by *M. rubra*. What prevents *M. scabrinodis* from replacing *M. rubra* entirely in this region? The answer to this question appears to lie in a difference of adaptations which gives *M. rubra* an advantage over *M. scabrinodis* in cooler moister sites of longer vegetation. The workers of *M. scabrinodis* have shorter legs and a build that fits them for crawling through dense vegetation and cracks in the soil. They are tolerant of, and resistant to, low humidities, and their nests, in which they remain all winter, are lined with heavily muddied walls. On the other hand, the workers of *M. rubra* have longer legs and a build that fits them for quicker movements over grass-herb vegetation. They can work in the warmest stratum in sunny weather. They are less tolerant of, and resistant to, low humidity. Their nests have little mud on the walls, and the summer nests are deserted in the winter. So although they select the same sorts of places for forming their nests, these species tend to forage in different places and, in addition, *M. rubra* will build its nests in cooler places than *M. scabrinodis* when forced to do so.

Antagonisms of this sort between different species of ants are not particularly uncommon. An extreme example is the Argentine ant *Iridomyrmex humilis* Mayr, which has been successful in a number of places in exterminating native species of ants, as for example in the Mobile area of Alabama [Wilson (54)] and in Madeira [Brian (11)]. Similarly the introduced fire ant *Solenopsis saevissima* v. *richteri* Forel is antagonistic to native species of ants in the area it has invaded in the states of Alabama, Florida, and Mississippi. Since its spread, the native ants *Solenopsis xyloni* McCook and *Solenopsis geminata* (Fabricius) and the Florida harvester ant *Pogonomyrmex badius* (Latreille) have become scarce in the invaded area [Wilson (54)]. The ant *Oecophylla longinoda* (Latreille), native of East Africa, has been destroyed in some coconut plantations by an introduced ant from India, *Anoplolepis longipes* (Jerdon), which replaces it in such plantations [Way (53)].

Brian (10) studied the foraging habits of four species of ants when a syrup was placed in positions accessible to all four. The syrup tended to become monopolized by one species, usually by that with the nearest nest, though, sometimes by the species which happened to find the syrup first. Occupancy of the food source produced a change in behaviour of the ants; they appeared to be more "possessive" and once the food was being used, other species appeared less willing to trespass.

Brown & Wilson (12) cite numerous examples of species of ants, frogs, and birds in which closely related species have overlapping ranges, and in their area of overlap the related species show more divergence in various characters than elsewhere. The variations referred to are mostly morphological, but there are also differences in some cases in physiology and behaviour. Brown and Wilson suggest quite logically that these differences have been evolved in the region of overlap because hybrids are at a selective disadvantage. Selection against hybrids is an effective mechanism for preventing gene flow between the two species. There are good grounds for supposing that natural selection will favour the fixation of characters which will preserve the integrity of the gene pool of each species. However, Brown and Wilson use this argument to support a statement that these are examples of "genetic fixation of specializations resulting in the avoidance of competition." They imply further that the differences between related species in the region of overlap arose as a result of competition between them. They define competition as "seeking or endeavouring to gain what another is endeavouring to gain at the same time"; the definition presumably implies, though it is not explicitly stated, that what is sought is in short supply so that there is not enough for both. Now it may be that there was a scramble or contest for resources in short supply in the initial phases of overlap of these populations, but nothing in the evidence indicates whether there was or not. There appears to have been selection for morphological and other differences making for sexual isolation between the overlapping populations. For this to happen we only need to postulate that the hybrids would be less

fit than the parents. Such selection could occur with or without competition [Birch (8)]. Nicholson (40, p. 171), on the other hand, presents a clear example of natural selection of a strain of blowflies, *Lucilia cuprina* (Wiedemann), which could produce eggs without protein in the adult diet as a result of intensive food shortage. This could be described as natural selection with competition.

Leafhoppers.—Six species of the *lawsoni* complex of the leafhopper genus *Erythroneura* feed and live on the sycamore tree *Platanus occidentalis*. Ross (43) studied the distribution and abundance of these six species on sycamores in the state of Illinois over a period of three years. His conclusion was that "all appear to have identical niches, the generations of the various species maturing synchronously in each locality, hibernating together and feeding in the same manner, often side by side on the same leaf." The only clear differences Ross was able to note between the species were differences in abundance. In the three years of study, *Erythroneura lawsoni* Beamer was the predominant species, then followed *arta* Beamer, *usitata* Beamer, *torella* Beamer, *morgani* De Long, and finally *bella* McAtee, which was quite rare in relation to the others. Ross found no indication that leafhoppers multiplied to such an extent that they ran short of food or space or that one species in any way influenced the abundance of the others. Trees with large populations of leafhoppers almost always supported all six species; whereas on trees with smaller numbers it was usually possible to find only *lawsoni* or *arta*. If one species were tending to exclude another, then one might have expected to find the reverse situation. Ross never did. The only evidence that leafhoppers were influenced by other species occurred when the tingid *Corythucha ciliata* (Say) was abundant on sycamores: then the leaves became brittle and dried out, and consequently few if any leafhoppers were found. Ross concluded that the six leafhoppers, though they used the same resources, did not influence one another's abundance.

Drosophila.—*Drosophila pseudoobscura* Frolowa, *Drosophila persimilis* Dobzhansky and Ebeling, and *Drosophila miranda* Dobzhansky are closely related species which as adults are readily collected together in the same places in Mather, California. Sokoloff (44, 45) studied the effect of crowding larvae of these flies in small vials of food. When each species is crowded with larvae of its own kind the mortality rose with increasing density until, at a density of 80 larvae per vial, none survived. Adults from crowded vials were much smaller than those which had come from vials where there was ample food. When larvae of *D. pseudoobscura* were crowded with those of *D. persimilis*, more of the latter than of the former survived. It seemed that the larvae of *D. persimilis* were better fitted to living in a crowd when both species were together.

In Mather the larvae of *Drosophila* can be found in "slime fluxes," (moist exudations of sap) on oak trees. The number of eggs and larvae in these slime fluxes is, however, very small, a few dozen and no more per flux. Yet in the same region adults can be trapped in enormous numbers; further-

more they are large and not like the small adults obtained from crowded vials in the laboratory. Very few flies appeared to have suffered from starvation during their development, judging from their size. In view of the large number of adults we might expect to find a large number of eggs and larvae in the breeding sites, but this is not the case. What is the reason for this? Sokoloff finds that a slime flux had quite an assortment of small animals living in it, such as larvae of insects and nematodes, and there are others which visit it for moisture. Adults of *Drosophila* appear to be attracted to the slime flux, probably by the fermenting smell. After arrival at the flux, the fly is soon disturbed by the activities of the larger insects and frequently will fly away. Several attempts may be made to find a suitable site on the flux for feeding or laying eggs. Some flies are caught by spiders. Even if a fly does succeed in laying its eggs, it lays only a few because its feeding or egg laying is continually disturbed by other organisms in the slime. Many of the eggs laid may not hatch but instead may be buried and drowned by the activities of other insects and nematodes. Larvae which do emerge may be destroyed by predatory larvae in the slime, and many succumb from other adverse influences such as cold and drying up of the slime fluxes. The outcome of all these influences seems to be that few eggs are laid and that from those that are, few larvae develop to adults. Those that do are well fed. Sokoloff says he found no evidence that would suggest "intraspecies or interspecies competition" between *Drosophila* in slime fluxes. Apart from components of environment such as weather, the low number of larvae in the breeding sites seems to be attributable to the interference of *Drosophila* by nonpredatory organisms and some predators in and around the slime flux. The example is particularly interesting because the two initial observations of abundance of adults and limitation of breeding sites for larvae might lead to the superficial conclusion that larvae must inevitably be crowded in the breeding sites. Birch & Battaglia (9) reported a similar lack of crowding of *Drosophila willistoni* Sturtevant in fruits in Brazil.

PREDATORS

The characteristics of predators in searching for their prey have recently been reviewed by Burnett (13). We have chosen to discuss here an outstanding study of predation made by Huffaker & Kennett (24) and Huffaker (23). This is one of the few studies in which numbers of predators and prey have been recorded over several generations of both.

Huffaker & Kennett (24) studied predation of cyclamen mite, *Steneotarsonemus pallidus* (Banks), by a predatory mite, *Typhlodromus cucumeris* Oudemans, in a series of experiments in both a greenhouse and in the field. Seventy-two potted strawberry plants which had been treated in January with an insecticide to destroy any predators were arranged into two groups in a greenhouse in April. By that time the cyclamen mite was present as a light infestation on the plants; the predatory mite was also present, but in low numbers. Half the potted plants were treated again with parathion to

destroy the predators. To the other half were added three predators per pot. From this time, for a year afterward, the numbers of predators and prey were counted at intervals of 11 days. In the course of the year the 36 pots which were intended to be free of predators did on two occasions have a few predators. When this was discovered, they were again treated with parathion. On the predator-free group of potted plants the cyclamen mite increased to some 60 mites per leaf, and after this large initial rise the numbers fell but rose and fell again twice in the course of the experiment. The mites were fairly common despite their fluctuation in numbers. By contrast, the numbers of cyclamen mites on the pots with predators never increased beyond a maximum of about eight mites per leaf. The numbers oscillated, as did the numbers of the predators. There was no doubt that the rareness of prey in these pots resulted from the presence of the predatory mite.

The experiment was repeated in the field with remarkably similar results. Plots of strawberries infested with the cyclamen mite had some predators added to them early in the fall. Similar plots nearby had no predators added. None of the latter plots developed a population of predators that year. Nor was there any appreciable movement of predators to these plots from those to which predators had been added. In the predator-free plots, the numbers of the prey increased until severe damage to the plants resulted. In the plots provided with predators in the fall, the prey did not increase in numbers sufficiently to cause any serious damage. Further observations on field plots indicated that for an economically successful control of the prey it was necessary that the predators be established early in the season. At that time the prey are normally scarce, and so Huffaker and Kennett have suggested that the economic control of the cyclamen mite might be more effective if both prey and predator are introduced early in the season to insure the establishing of the prey population.

During five years of study of the cyclamen mite and its predator, the extermination of either predator or prey on any small group of plants or even perhaps on a single plant seems never to have happened. This may be explained by certain qualities of both the predator and its prey. The predator has difficulty in finding all the prey, especially those which are concealed in such places as leaf crevices, so that some prey are probably always protected. Secondly, the predator itself is to some extent protected during periods of scarcity of prey by its ability to use honeydew as food. Another feature which reduces the probability of extermination of prey or predator is the diversity of refuges in which the former live. The provision of refuges has seemed to be an essential aspect of laboratory models, if the prey is not to be exterminated by the predator. It was certainly a characteristic of those populations which Huffaker and Kennett studied, though one cannot be sure that this was the reason why the prey were not exterminated in their experiments.

The extermination of prey in so many laboratory models which have been studied may be associated with the small size of the model, which virtually ensures that the predator does not search for the prey but, in fact,

has little chance of avoiding it. Huffaker (23) designed a number of laboratory models which gave both predator and prey varying amounts of dispersal by increasing the size of the model and providing barriers to dispersal, but without providing refuges for prey or predator. He used as prey the phytophagous mite *Eotetranychus sexmaculatus* (Riley) and as predator the mite *Typhlodromus occidentalis* Nesbitt. The essential differences between his various models lay in the arrangement and number of oranges which served as food for the prey and of rubber balls the size of oranges. When few oranges were used, whether they were close together or sparsely distributed in space, the prey was eventually annihilated by the predator in a matter of a month or so. But Huffaker did succeed in elaborating one experimental arrangement of his oranges in which both prey and predator persisted in a succession of three oscillations covering six months. The essential features of this design seemed to be the large number of oranges used (120 instead of four), their regular arrangement on a flat surface, and perhaps more important the existence of barriers of Vaseline and other impediments to movements of the mites. This design must have reduced the dispersal of the mites, and it evidently gave an advantage to the prey by reducing the chances of a predator finding it. The predators and prey in this experiment "played a game" of hide and seek which lasted for some six months before all the prey were found. It is a nice model of the sort of situation Andrewartha & Birch (4) envisaged as being important in natural populations of predator and prey. When to the incapacity of predators to find all the prey because of less than perfect dispersal or hunting one other component (deliberately excluded from Huffaker's experiment) is added, namely protective refuges, then the continued coexistence of prey and predator is more readily understood.

A PLACE IN WHICH TO LIVE

REQUIREMENTS OF TSETSE FLIES²

A feature of the ecology of tsetse flies is their particular requirements for what Andrewartha & Birch (4) have called "a place in which to live." Much advance has been made in recognizing the particular sorts of vegetation which a species haunts, but it has not usually been possible to identify the components which make such places suitable for tsetse flies. The following examples have been taken from Bursell (14), Buxton (18), Glover *et al.* (21), Jackson (26), Nash & Page (37), and Nash & Steiner (38).

Many types of woodland appear to be too dense for *Glossina morsitans*. The type of vegetation known as "miombo" in East Africa may be so thick that it excludes this species. The southern boundary of *G. morsitans submorsitans* Newstead in West Africa is a barrier of denser vegetation which limits spread of this species over a breadth of country 100 miles across. An

² Dr. J. P. Glasgow and Dr. E. Bursell of the East African Trypanosomiasis Research Organisation, Shinyanga, Tanganyika, very kindly checked this section.

experimental densification of an area was carried out at Shinyanga in Tanganyika in country occupied by *G. swynnertoni*. When fire was excluded from a block of thornbush, there was a noticeable increase in thicket within two or three years and the numbers of *G. swynnertoni* fell by 90 per cent in three years, compared with control blocks in the same neighbourhood [Buxton (18, p. 555); Jackson (26)].

The same effect, namely drastic reduction in the density of the population of species of the *morsitans* group, has been achieved by the widely practiced "discriminate clearing." This method is to recognize the type of place where male flies are to be found (females are mostly difficult to find) and then to clear or partially clear them to reduce the cover [Jackson (26)]. Bursell (14) found that in a recent extension of *G. morsitans* in southern Tanganyika the flies were common in short-grassed glades where there was a double canopied interzone between grass and woodland. They were not common in the woodland itself. Clearing of the upper canopy in this interzone caused a drastic reduction in the density of flies.

Another example of this is provided by the work of Glover *et al.* (21) in Northern Rhodesia. Their surveys of the incidence of *G. morsitans* led to the recognition of two sorts of places where tsetse flies tended to be found in large numbers. One was the small valley known as a "kasenga." Typically a "kasenga" starts between high hills as a narrow but flat-bottomed course and becomes a rocky ravine. The upper flat-bottomed section supports a fair growth of trees, and the adjacent bushlands are also well wooded. The second sort of place favoured by the flies was also a valley but distinguished from the first by its steeper stony sides and a sandy stream bed at the bottom, with frequent poorly grassed patches supporting low growing *Vellozia*. These valleys were also surrounded by woodland. After these favoured sites had been recognized and an estimate of the apparent density of the flies had been obtained, the trees in the valleys and on the edge of the surrounding woodlands were felled. In both types of valley the tsetse population collapsed dramatically—so much so that the authors refer to the results of clearing as "extermination." In a typical case before the clearing was done, 50 flies were caught per 10,000 yards traversed. In the five successive years after clearing, the numbers were 35, 15, 1.7, 0.1, and 0.

When the tsetse fly was on the decline but still relatively high in numbers, an uninseminated unteneral female fly was captured in one of these valleys, an observation never before made in Tanganyika. In the following year, 12 uninseminated females out of a total of 136 unteneral females (i.e., nine per cent) were captured. In the third year, the percentage had risen to 29. Evidently the sexes were beginning to fail to encounter each other quite soon after the clearing had been done, and the proportion of females which failed to find a mate increased in successive years. Hence, the final disappearance of the flies may have been brought about when the population reached a critically low level at which the chance for a female to find a mate was too small for the maintenance of a population. The authors were un-

able to account for the favourable qualities of the valleys. They concluded, "It must be confessed that we do not know what vital role these valleys played in the ecology of the flies, we only know that cutting down the trees in and immediately around them put them out of action."

In the Lake Province of Tanganyika there are extensive low-lying plains which carry a pure stand of the gall-bearing *Acacia*. The plains are surrounded by slightly higher areas of thornbush. The thin and open vegetation of the plains is unsuitable as a breeding place for tsetse flies. On the other hand, the thornbush which surrounds these plains is a permanent breeding place of *G. swynnertoni*. The boundary between thornbush and plains is not sharp since the thornbush tends to penetrate the plains and form islands of bush. Tsetse flies wander out through this network of denser vegetation and thus invade the plains, where they feed on cattle. The flies use the plains for feeding, though they cannot breed there. However, when the connection between breeding site and feeding site is cut by clearing away the connecting network of thornbush, the flies in the plains disappear. This fact has been most effectively used in reclamation of the plains [Buxton (18, pp. 289, 572); Jackson (26)].

In the northern territories of the Gold Coast, climate is unfavourable in the dry season for *Glossina palpalis* (Robineau-Desvoidy) and *Glossina tachinoides* Westwood. These tsetse flies are then restricted to "fly belts," i.e., associations of particular species of trees which form a dense tunnel-like shelter along the edges of water courses, completely closing them in if they are narrow. The microclimate in these fly belts is both cooler and moister than in the country beyond. When the bush along these streams is destroyed, the populations of *G. palpalis* dwindle dramatically—that is, in the drier and more northerly parts of its range in West Africa [Buxton (18, pp. 294, 299)]. Similarly *G. tachinoides* has been nearly exterminated over about 1000 square miles by the eradication of thicket-forming species of riverine plants [Jackson (26)].

Nash & Page (37) made a six-year study of the distribution of *G. palpalis* in Northern Nigeria, where it is confined to riverine vegetation. They concluded that this tsetse fly needs the vertical insulation from the sun provided by high canopy, the lateral insulation from the sun provided by creeper curtain or steep stream banks, and a free flight long the stream bed so that in the hot, dry season it can "fly from crossing place to crossing place in search of food." This led to their suggestion that flies might be eradicated from these riverine habitats if the stream bed were blocked by felling the trees which formed the canopy so that they fell into the stream. The destruction of both the vertical insulation and the free flight line might then make the riverine habitat untenable in the dry season and force the flies to seek food in the adjacent savannah, where the weather is lethal in summer. Nash & Steiner (38) reported the results of just such an experiment in "obstructive clearing," as they called it, of three and a half miles of the Kiyu River. There was a great reduction in numbers of tsetse flies, compared with control

areas of the stream. One month before clearing, 41 per cent of the flies were "hungry"; three months after clearing, 79 per cent were "hungry." The flies disappeared completely during the dry season, but there was reinvasion from neighbouring areas during the wet season. Nash and Steiner's results did indicate, however, that if the cleared areas were isolated obstructive clearing would result in eradication of the flies.

Buxton states that the distribution of most species of tsetse flies is discontinuous, since patches of vegetation which provide the necessary conditions for survival are often separated by areas in which suitable vegetation does not occur. This patchiness will be aggravated when the species needs to frequent more than one sort of vegetation zone, as in those areas where the fly apparently has breeding grounds which are distinct from feeding grounds [Jackson (27)]. These characteristics of the sorts of places where tsetse flies live accentuate the importance of the capacity for dispersal in the life of tsetse flies.

GENETICAL ASPECTS OF POPULATION ECOLOGY

The genetic plasticity of species [see Andrewartha & Birch (4); Dobzhansky (20)] means that the ecologist should no longer study a species without reference to its genetical variation in both space and time. The genetical composition of a species is now known to vary in relation to changes in the components of environment.

RACES OF *ORNITHODORUS MOUBATA*

The argasid tick, *Ornithodoros moubata* (Murray), which is a vector of relapsing fever, is widely distributed in East Africa. It occurs in both hot, arid regions and cold, wet regions. Walton (50, 51, 52) made an intensive study of the distribution and abundance of this tick in Kenya and Tanganyika in over 4600 huts where it lives. The extraordinary diversity of climates in which *O. moubata* is able to survive and multiply constituted a real puzzle until Walton's work revealed that there are at least four distinct races within the total distribution. One of the fascinating features of his study was that he was led to this discovery by comparison of the abundance of ticks in regions of very different climates. This showed up differences in tolerance to humidity and temperature of populations from different geographic areas. It was not until a late phase of his investigations that any morphological differences became apparent. These, moreover, are so slight that they can only be discriminated by observing the shape of engorging ticks having their first meal. Walton's investigations strongly suggest that there are four races of ticks differentially adapted to their special habitats in East Africa.

Form A feeds primarily on man and is found only at high altitudes in areas with a cool, wet climate. It was the observation of decrease in abundance of the tick with decrease in altitude on high mountains in Kenya which led to the discovery of Form A. It does not feed on domestic fowls, even when

they are present in the huts. It occurs in greatest abundance in places where the relative humidity inside the huts is 86 per cent and the temperature ranges from 68° to 78°F. It is absent in regions where the relative humidity in huts is consistently over 90 per cent and may not occur in places where the relative humidity is consistently less than 75 per cent. This form lays a large number of eggs compared with the other forms. It can be identified by the flattened oblong, ovate shape assumed when feeding for the first time on a rabbit's ear.

Form B feeds on domestic fowls in African huts. In the absence of fowls it is rare. It occurs in places where the air in the hut is moist and the temperature is warm to hot. It is most abundant when the mean temperature of the hut was 78°F. and the mean relative humidity 83 per cent. But it has been found in places where the temperature ranges from 69° to 84°F. and the relative humidity ranges from 77 per cent to 90 per cent. In comparison with Form A, it is adapted to moister and warmer places. It lays fewer eggs and can be identified by its peculiar posture in feeding, when the posterior portion of the body becomes distended while the anterior end maintains prolonged waves of contraction.

Form C feeds mainly on wart hogs and is found most frequently far from human habitation and characteristically in the burrows of wild animals, especially those of wart hogs and porcupines. It lays fewer eggs than Form A and more than Form B and can be identified by its reluctance to feed on a rabbit's ear. When it does, however, it assumes a characteristic semispherical shape. The mean temperature and related humidity of its haunts were 76°F. and 72 per cent.

Form D appears to feed on man and domestic fowls with equal frequency. It occurred in huts in hot and arid regions and was found most abundantly in the hottest, driest huts of all those examined. The mean temperature and relative humidity of these huts were 86°F. and 43 per cent, respectively.

LIGHT AND DARK RACES OF THE FIRE ANT

A dark race of the South American fire ant *Solenopsis saevissima* v. *richteri* Forel, was introduced into the port of Mobile, Alabama, around 1918. By 1949 it had spread to Florida, Mississippi, and Louisiana and a long way northward into Alabama. Coinciding with the rapid spread which seemed to take place in the 1940's, there was a change in the colour of the ant. The original dark phase became almost completely replaced by a light colour phase, except at the periphery of the distribution where some dark ants still remained. The two phases differed also in size and in the appearance and proportions of their nests. The differences are genetic and probably result from a number of alleles. The origin of the new form is not known; it may have arisen by mutation, or it may have arrived in a second importation of ants from South America. However, over a period of about 20 years there has been almost a complete replacement of the original dark variant by the new light one (54).

GEOGRAPHIC RACES OF THE QUEENSLAND FRUIT FLY

During the last 50 years the Queensland fruit fly, *Dacus tryoni* (Froggatt), has evidently spread from its tropical home in the rain forests of Queensland in Australia southward into the states of New South Wales and Victoria, a distance of some 1500 miles. Originally living in the fruits of rain forest trees, the fly has spread into cultivated fruits which are now grown in many districts all along its route. Without fruit it could not have spread. But in addition to this, the spread south has involved an evolutionary adaptation

TABLE I*

THE INNATE CAPACITY FOR INCREASE EXPRESSED AS THE FINITE RATE OF INCREASE PER FEMALE PER WEEK OF FOUR GEOGRAPHIC STRAINS OF THE QUEENSLAND FRUIT FLY *D. TRYONI* AT THREE TEMPERATURES. THE POPULATIONS WERE COLLECTED IN 1957

Temperature °C.	Strain			
	Cairns	Brisbane	Sydney	Gippsland
20†	0.789	1.046	1.268	1.216
25‡	1.582	1.598	1.546	1.680
30§	2.023	1.883	1.631	1.929

* Bateman (5).

† Comparisons are significant at $P = .05-.01$, except Sydney and Gippsland.

‡ Comparisons are not significantly different.

§ Comparisons are significant at $P = .01$, except Cairns and Brisbane, Gippsland and Cairns, and Gippsland and Brisbane.

of the fly to progressively cooler latitudes. This has been proven by a comprehensive series of experiments by Bateman (5), who studied populations of the fruit fly from four geographic areas in a transect of 2000 miles from north to south along the east coast of Australia. For each of these populations he determined at three temperatures the age schedule of mortality (inverse of the life table), the rates of development of the immature stages, and the age schedule of egg laying. From these he estimated the innate capacity for increase (r_m).

Table I shows the innate capacity for increase (expressed as a rate of increase per female per week) for the four populations from Cairns (northernmost population), Brisbane (subtropical), Sydney, and Gippsland (southernmost). This information was obtained from populations collected in the field in 1957; the results are similar to those obtained in the previous year. The Cairns strain from the tropical north has the lowest innate capacity for increase of the four strains at 20°C. and the highest at 30°C. The Sydney strain (1500 miles south) has the highest value of the four strains at 20°C. and the lowest at 30°C. The values for Brisbane fall nicely in between these

two. This means that the strain from Cairns could survive and multiply rapidly in areas which would be too hot for rapid multiplication of the Sydney strain. The Sydney strain, on the other hand, could survive and multiply rapidly in areas which would be too cold for rapid multiplication of the strain from Cairns. The innate capacity for increase of the southernmost strain from Gippsland was about the same as Sydney at the low temperatures and higher at the high temperature. This result at first sight seems to be in conflict with the requirements of adaptation to the cooler south. But there is no anomaly here. High temperatures occur more frequently in the south (Gippsland) than in Sydney, so there would be an advantage in being able to increase at high temperatures there. Furthermore, Bateman showed that pupae and adults of the Gippsland strain were more resistant to both extreme low and extreme high temperatures than the northern strains. These results suggest that selection there has been in relation to extremes of weather. The fly in Gippsland may be at its adaptive limit, since it has never been abundant there and for the last two years, 1957 and 1958, it could not be found at all.

The main evolutionary changes which evidently occurred in altering the innate capacity for increase were in the number of progeny and in the life expectancy of adults at different temperatures. For example, if we compare the Cairns strain with the Sydney strain we find that at 20°C. the number of progeny is greater for the Sydney strain at all ages of the parent female and that this strain starts laying eggs several weeks earlier. In the tenth week the Sydney strain lays nearly 30 times as many eggs as the Cairns strain. By the twentieth week it lays about twice as many eggs. This may, indeed, be an illustration of the principle that an increment in the innate capacity for increase can be most readily obtained by enhancing the rate of egg production early in the female's life. Eggs laid later make a smaller contribution. The survival of adults of the Sydney strain is also greater at all ages than that of the Cairns strain at this same temperature. The Sydney climate has evidently imposed a selection for a temperature such as 20°C., whereas in Cairns selection at such a temperature could hardly occur. Altogether Bateman's results demonstrate a beautiful adaptive relationship between innate capacity for increase and climate. We think this is the most complete study of ecological differences between races yet made. Adaptive differences between four geographic races from different climatic regions have also recently been discovered for *Drosophila pseudoobscura* and *Drosophila persimilis* by Pittendrigh (41).

A colour variant described as *Dacus tryoni neohumeralis* Hardy (which has brown humeral cali instead of the yellow of *D. tryoni*) occurs sympatrically with *D. tryoni* in the tropical parts of Australia. Bateman (5) found that of flies reared from guavas in Cairns 90 per cent were *D. tryoni neohumeralis*, up to 3 per cent were hybrids, and the rest were *D. tryoni*. In Brisbane only 7 per cent were *D. tryoni neohumeralis*. In Sydney *D. tryoni neohumeralis* does not exist, nor is it present south of Sydney. These observations suggest that the

variant *neohumeralis* is adapted to a tropical climate. It has not spread into the subtropics and temperate regions with *D. tryoni*. In order to test whether there were selective differences between the two variants at different temperatures, Birch (unpublished) measured the change in frequency of the two types kept in population cages for many generations at 25°C. and at 20°C. When populations were crowded, selection was in favour of *tryoni* at the relatively low temperature of 20°C. but in favour of *neohumeralis* at the higher temperature of 25°C.—results which are completely in accord with the field observations that *neohumeralis* is common in the tropics and absent in the temperate regions of Australia. There is also evidence that the two variants have different host-fruit preferences. The small amount of hybridization in the field as well as the incompatibility of the cross one way indicates considerable sexual isolation between the two forms [Bateman (5)]. The work on the Queensland fruit fly has thus revealed climatic races and race formation almost to the point of speciation. Recognition of differentiation of this sort between races of insects will almost certainly become more common as entomologists turn their attention more toward this type of investigation.

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SAMPLING INSECT POPULATIONS^{1,2}

BY R. F. MORRIS³

Forest Biology Laboratory, Fredericton, New Brunswick, Canada

INTRODUCTION

This will not be a review, in the usual sense of the word, because the subject is much too broad to be covered in the allotted space. Almost every serious attempt to study natural or applied control in the field has involved population sampling, in one form or another, so that a complete review of sampling methods would have to cover most of the extensive literature dealing with field studies of insects. The principles of population sampling, of course, are universal and need not be reviewed here. They have been adequately treated in a number of recent textbooks, among which those of Cochran (21), Hansen, Hurwitz & Madow (47), Schumacher & Chapman (81) have proved most useful in connection with my own sampling problems. In the application of these principles, however, the great diversity in the life cycles and habitats of different insect species, and in the particular objectives of different field studies, has led to an equally great diversity in technique.

Therefore, instead of presenting a complete bibliography I shall attempt to appraise the present status of insect population sampling, with particular attention to its objectives, and to suggest logical subdivisions of the subject, any one of which might well provide sufficient material for subsequent and more complete reviews. In discussing designs that have been used in insect sampling, reference will be made to only a limited number of papers that most conveniently illustrate the main points. If these examples lean rather heavily toward forest insects, it is only partly a result of my greater familiarity with forest insect sampling, and perhaps mainly stems from the fact that the greater stability of the forest environment has encouraged the development of more sophisticated sampling designs for long-term studies of natural population dynamics. The frequent disturbances to agricultural crops and agricultural soils have been less conducive to such studies. Further, having taken liberties with "review" it will be no more than consistent to take liberties with "entomology" by drawing attention to sampling designs in fish and wildlife research, particularly for those aspects of population work where progress has been more rapid than in entomology.

¹ The survey of literature pertaining to this review was, with a few exceptions, concluded in January 1959.

² Contribution No. 542, Forest Biology Division, Research Branch, Department of Agriculture, Ottawa, Canada.

³ I am very much indebted to the following workers who brought useful literature references to my attention in the course of correspondence: A. H. Strickland, who maintains an extensive card index on sampling, O. W. Richards, H. Klomp, M. E. Solomon, J. Franz, V. Skuhravy, T. R. Southwood, W. Tischler, S. Iwao, G. B. Oakland, and K. E. F. Watt.

There appear to be no earlier reviews that are sufficiently comprehensive to serve as a logical starting point. The nearest approach is that of Chauvin (18), who devotes 15 pages to this subject in his excellent "*Réflexions sur L'écologie Entomologique*." Chauvin's treatment is concerned largely with methods of collecting insects from the soil, the herbaceous stratum, trees, water, and air, and only the last two pages deal with problems concerning frequency distribution and sampling design. Strickland (93) prepared a review on insect sampling for the Commonwealth Entomological Conference in 1948, but unfortunately his long and carefully classified bibliography was not printed in the report of the Conference. De Gryse (26) described the population work on forest insects up to 1934, but most of the important advances have been made since that time. While presenting a sampling design for the spruce budworm, *Choristoneura fumiferana* (Clemens), Morris (67) reviewed the principal North American work on forest insect defoliators between 1934 and 1955. Other authors have reviewed restricted aspects of sampling, particularly the methods of collecting insects from different media, and such works will be cited in later sections. Recent textbooks on ecology seem to devote more and more space to population problems and to sampling, viz., Allee *et al.* (1), Andrewartha & Birch (2), Odum (72), and especially MacFadyen (60). The entomologist will find these of interest, particularly as a source of broad reference material to biological sampling in general. To design a sampling program from such general summaries, however, would be as inadvisable as designing one after reading the chapter or two on sampling in the standard statistical handbooks.

OBJECTS AND METHODS

Population sampling has been used for many different purposes ranging from extensive surveys, designed generally for the prediction of damage, to very intensive studies of population dynamics on a small plot or two. The distinction between extensive and intensive sampling is a helpful one because it determines sampling design to a large extent. However, it is by no means a hard and fast distinction and the same basic data on frequency distribution, major sources of variance, and optimum size of sample unit are required for either extensive or intensive sampling. Thus, basic data obtained for intensive sampling of *C. fumiferana* were equally useful in designing sequential sampling methods for the survey of hundreds of square miles of forest (66).

Another useful distinction is that between the sampling of population density and the sampling of other attributes of population such as the stage of development, the degree of infection by parasites or disease, or the rate of mortality. When the main object of a study is concerned with such an attribute, relatively simple methods are often sufficient.

The purpose of the present section is to review some of the main objectives for which sampling has been used by entomologists, along with the general methods that seem to be appropriate for each. The importance of

clearly defining the objective in advance cannot be overemphasized (1, 81). Sampling has no intrinsic merit but is only a tool which the entomologist should use to obtain certain information, provided there is no easier way to get the information. He should keep his objective in mind at all times and refuse to be hypnotized by the elegance of modern sampling design. The criteria by which an insect sampling project should be appraised are these: Was the objective clearly defined? Could it have been satisfied by easier means than population sampling? Was the sampling design selected the most appropriate one for the objective, and was the optimum allocation of sampling resources determined? Where the objective is concerned with population dynamics, Watt (104) has correctly stressed that the type of mathematical model to be used in analyzing the data should also be chosen in advance since it determines the type and amount of data required as well as the sampling design.

Collecting.—Collecting is used when the objectives do not require the determination of population density but only such attributes as species composition, developmental stage, or degree of parasitism. It is helpful to distinguish collecting from population sampling, although the division between them is often indefinite. Even the taxonomist during his collecting has some appreciation of effort expended, and on the basis of this he rates species as rare or common. Thus collecting, with appropriate refinements, can provide a useful index of population density, and "catch per unit effort" data are used very effectively in this way in fisheries research (11, 45, 78). Even if such an estimate is not desired, collecting must often be based on the same principles of representativeness that apply to sampling. The adults of some parasites, for example, prefer to attack host larvae in the tops of tall trees (63), so a very poor estimate of percentage parasitism would be obtained by collecting only the host larvae that can be reached easily from the ground.

Experimental populations.—Experimental field populations can often be used in place of sampling when the main object is not to estimate population density but rather the rate of mortality under specified conditions. A known cohort of individuals is either discovered in the field and isolated, or stocked out in the field in marked locations, and examined periodically to determine the rate of mortality. This technique is used effectively for evaluating the effects of natural enemies on citrus pests in California, where the object is simply to determine whether the mortality of the pest is higher when the predator is present than when it is absent. Fleschner (37) recently reviewed this work, along with different methods for excluding predators from experimental populations, and concluded that removal by hand is more dependable than the use of sleeve cages, selective insecticides, or biological methods using ants. The work of Franz & Karafiat on *Adelges piceae* (Ratzeburg) attacking the trunks of fir trees in Germany is similar in principle and involves in some cases the successive plotting of individual aphids by special apparatus (38, 39). Dowden *et al.* (29) stocked small trees with known populations of de-

foliating larvae and used cages of different mesh to exclude birds from some, and both birds and parasites from others. Sometimes the object is not so much to apply a treatment as to measure the effects of natural mortality on a known population, especially when the population level in the field is too low to permit reliable estimates of mortality by the successive sampling of an unknown population. Graham (43) stocked sawfly cocoons in the litter at marked positions in the forest in order to learn how many were attacked by small mammals each year; this technique has been adopted for pest insects by other workers (17) and for parasite puparia, to learn what proportion of the puparia may be expected to produce adult parasites (57). The accumulation in the soil of cocoons or pupal cases of former generations often makes more general collecting or sampling unsuitable for this purpose. Itô & Miyashita (50) stocked cabbage plants with known numbers of armyworm eggs and examined the insects at successive intervals of four days to measure dispersal and mortality rates.

What this method really amounts to is, first, a very purposeful selection of the sample and, then, continued use of this known sample to reduce extraneous sources of variance in answering specific questions of limited scope. Where there is any intention to elucidate the mechanisms of interaction between prey, predator, and other factors, or the role of population density in natural control, more elaborate methods are necessary. As a means of assessing the rate of mortality the method is suitable for sessile or dormant stages. The caging or isolation required to confine active forms to known positions introduces an unnatural element. When experimental populations are stocked in the field to assess natural mortality rates, the density of stocking in relation to the density of the natural population is also a very important consideration.

Population class.—Not infrequently the entomologist makes extensive surveys designed to estimate mean population density at numerous points, but ends up by combining his results into two or three population classes such as "light," "medium," and "heavy," or even more simply "areas requiring treatment" and "areas not requiring treatment." This represents a great waste of effort and is an example of inappropriate design. When it is clearly recognized in advance that the objects of the survey demand only the establishment of population class, sequential sampling offers an appropriate method that will greatly reduce the cost or permit many more plots to be examined for the same cost. It was developed for quality control during the last war (101) and has been used successfully since 1950 by a number of biologists. Oakland (71) first applied it to a fisheries problem, and through his influence Stark (90) and Morris (66) designed sequential schemes for extensive surveys of certain forest insects in Canada. It is unnecessary here to review this work or to describe the method in any detail because an excellent review has already been presented by Waters (102), in which he gives the appropriate sequential formulae for normal, Poisson, binomial, and negative binomial distributions. Briefly, the efficiency of the

method comes mainly from the absence of a fixed sample size. Samples are drawn in sequence, and on a plot where population density falls definitely in one class or another, one or two samples will often suffice; on a plot that is near the borderline between two classes, more samples must be drawn to place it in one class or the other. Sequential schemes have now been developed for most of the major forest defoliators in North America, and it will be surprising if the method is not rapidly adopted in other fields of entomology and in other countries.

Population index.—A population index may be defined as any sort of measurement or count that is related to population density. Common examples are catch per unit effort, either by hand or in various types of traps, the amount of frass dropping from trees, or the amount of damage to host plants resulting from insect feeding. A population index, by itself, is of relatively little value because almost invariably it is related to other factors in addition to density. The number of moths taken in a light trap in a given period, for example, depends not only on the population available for trapping but also on activity, which in turn depends on meteorological factors. However, once we know enough about a species to make a correction for the influence of other factors, the index can become a very useful tool. It may be converted to a *relative index*, which is a measure of the relative populations available for trapping in different periods or different areas, or even to an estimate of absolute population density. Population indices and mathematical techniques for their conversion and analysis have been developed to a much higher degree in fish and wildlife research than in entomology because fish, mammals, and birds do not lend themselves so readily to direct population counts as do insects. Thus census techniques have been developed on the basis of such indices as catch per rod-hour, commercial pelt records, frequency of prey remains in stomachs of predators, and counts per unit area or distance of shed antlers, droppings, songs, tracks and trails, nests, beaver lodges, muskrat houses, etc. (1, 79). Because many insects lend themselves to more direct methods, the entomologist is often prone to adopt them at once, without considering whether his objectives could not be achieved by the use of indices. And having adopted direct counts, he often finds that they are so time-consuming that they restrict his work to one or two small plots; whereas by using suitable indices he could have covered many times the area with the same expenditure of time. Graham (44) appears to be of this opinion: his textbook on forest entomology lists four methods for using indices and only one for using direct counts.

The measurement of frass drop as an index of forest insect populations has been developed and used rather widely in Germany. The literature has been reviewed by Morris (65), who developed methods for converting the index to estimates of both relative and absolute abundance of *Diprion hercyniae* (Hartig). Unfortunately, the frass of some forest defoliators does not fall freely enough to permit use of the method (67, 73). The degree of defoliation or other types of damage caused by insect feeding is also useful,

particularly when it can be recognized from the air and mapped rapidly over large areas, and this technique has recently been reviewed by Balch *et al.* (4). Various authors, such as Ewan (32), have worked out regressions between feeding or oviposition damage and actual insect numbers. A rather ingenious technique for using the drop of larvaevorid maggots in measuring spruce budworm populations is described by Bean (10). The budworm pupates on the tree, so maggots dropping to pupate in the soil are caught on large sheets of cotton under the tree, and, if the percentage parasitism of the host population is known, the total number of host larvae in the tree can be calculated. Mechanical methods of transportation can be used to obtain rapid indices of population for some species over large areas. The nests of colonial web makers along roadsides are readily counted from a vehicle (97), or the number of locusts flying up from grassland in front of a Land Rover or Swamp Skipper (80).

Odum (72) points out that the conversion of indices to actual population is a very important job for population ecologists and one that generally is yet to be done. This is true, but some useful advances have been made. In this regard the work of Davidson & Andrewartha (24), in which partial regression methods were used to explain the variance resulting from weather in successive captures of a thrips on roses, is a classical example. The same type of analysis is useful for sweep net data (49). The more involved techniques developed in fisheries research for similar catch per unit effort data cannot be reviewed here, but the entomologist who considers use of this approach will find leads to the literature in Beverton & Holt (11), DeLury (27), Gulland (45), Ricker (78), and Scattergood (79). One of the few applications of such methods in entomology is presented by Kono (59), who tested an equation for relating insect population in a given area to time and collecting efficiency when the insects were collected at random by hand.

Recapture method.—Trapping can be used to provide estimates of absolute population if captured specimens are marked, released, and subsequently re-trapped. The proportion of marked individuals in the catch permits a calculation of the total population available for trapping. This technique, often called the "Lincoln Index," has been used very extensively in fish and wildlife work and useful references to the rather extensive literature on the subject are available in the ecology textbooks cited above. Jackson's series of papers on tsetse flies, reviewed by Andrewartha & Birch (2), appears to present the first application of the method to insect populations, and in recent years this approach has been applied to many other insects, including butterflies (30), grasshoppers (77), locusts (28), grain beetles (48), lace bugs (89), soybean beetles (53), and even ground beetles caught in pitfall traps (86). The mathematical aspects of analysis have been highly developed, as reviewed by Ricker (78) and Beverton & Holt (11), and it is possible to estimate recruitment or depletion of population between successive samples. Recent developments in techniques for marking insects have made the recapture method a practical tool for the entomologist and its use is likely to become much more general.

Absolute population.—Despite the usefulness of indirect methods, there are some objectives that can be met only by direct counts of insects on sample units of known size. It must then be decided whether the estimation of population intensity or absolute population is required (67). The former is an expression of numbers in terms of food supply or living space. (Some authors have used "population density" in this sense, but more often density is used in a very general sense to express no more than would be implied by "population level" or "population numbers.") Absolute population is the expression of numbers in terms of a unit that does not vary, particularly an area unit of the earth's surface. Annual or seasonal changes in the amount of foliage produced by plants, the depth of organic litter on the ground, or the flow of water in streams give rise to changes in population intensity which reflect only a change in food supply or living space and not a true change in population. For example, the number of shoots produced each year by a tree may vary considerably, so the expression of spruce budworm population per shoot, although useful in predicting damage, suffers variations that are not true population changes (67). Gabbutt (40) presents an interesting comparison of the two methods of expression for the fauna of oak leaf litter, showing that seasonal population trends are quite different for numbers per unit area and for numbers per weight of litter. Conclusions on the response of bird predation to changes in the populations of insects attacking pine cones depend upon whether insect population intensity per cone is used, or absolute population per area of forest, since the annual production of cones is an important variable (42).

It appears that nearly all entomologists who have attempted careful long-term studies on population dynamics have found the absolute unit essential. It affords a stable unit for frequent sampling to develop life tables, provides a common reference point for a species that feeds on foliage but pupates in the ground, and permits direct comparisons between the population of the insect and that of its vertebrate predators. For foliage feeders, the conversion is generally achieved by sampling on the basis of a foliage unit and subsequently determining the number of such units per unit of land area. Space does not permit a comparison of the techniques used for this purpose by Ives (52), Klomp (57), Morris (67), Richards & Waloff (76), and Schütte (82). For deciduous species of trees, Tinbergen (95) suggested an interesting method based on the measurement of leaf fall in the autumn.

MECHANICS

I use the word "mechanics" to embrace all the tedious but necessary procedures involved in operating traps or collecting samples, extracting, counting, and recording insects. Perhaps use of this term is a bit wistful, for, although these procedures become highly routine, the development of really mechanical methods has been slow and most of the work must still be done by human eyes and hands. Entomologists have given much attention to mechanics, particularly to methods of trapping and extracting, and a very large majority of the papers examined in connection with this review were

concerned with mechanics to the exclusion of design or other aspects of sampling. This is not to be deplored because design is often limited by mechanics and all too often slow and cumbersome extraction methods contribute the major portion of the cost function in calculating optimum allocation of sampling resources (67).

There is probably no need to say that the mechanics of sampling a given species depend upon its life history and habits, which determine among other things the best time to sample. The factors affecting timing have been reviewed elsewhere (67). When the objectives require only one population estimate per generation, a quiescent, easily-reached stage is usually selected. Thus, surveys of pupae hibernating in the forest litter have been a standard practice in European forests for many years (83). When the objectives require frequent sampling during each generation, it is often necessary to sample active stages that are changing rapidly in population. This demands very careful timing, aided sometimes by phenological indices, and if many plots must be sampled on the same day it may be necessary to preserve the samples by chemical means, by deep-freezing, or, in the case of sessile forms, by photography (39), for subsequent extraction or counting. If egg or pupal sampling is delayed until emergence is over, useful data on mortality factors affecting these stages is often provided (67). Similarly, when larvae moult in feeding galleries or make new cases or cells in each instar (26), the population reaching each instar may sometimes be determined from one sample taken at the end of the larval stage.

Space is not available for a review of the extensive literature on collecting and extracting methods, and the reader is referred to Chauvin (18), MacFadyen (60), Strickland (93), and Unterstenhöfer (98) for a broad coverage, to Guyer & Hutson (46) for aquatic insects, to Solomon (88) for stored products insects, and to the various authors of *Soil Zoology* (55) for soil fauna. A classification of the numerous and varied techniques within three logical steps is suggested below.

I. Collecting

- A. Direct—by sweep nets or similar devices employing motion; by various types of traps employing wind, light, sound, suction, sticky substances, natural or chemical attractants, pit falls, electricity, or baffles; or by the application of insecticides.
- B. Indirect—by collecting a sample of the insect's medium (foliage, soil, water, air) and later extracting the insects; or by collecting the gizzards or stomachs of the insect's predators.

II. Extracting

- A. Hand-picking (used for most foliage insects).
- B. Mechanical methods (soil or grain sieves, flotation methods, leaf-brushing or imprinting).
- C. Chemical methods (fumigants, repellants, anesthetics).

- D. Behavior-type methods (light, heat, or similar factors utilizing the insect's behavior to extract it from the sample).

III. Counting

- A. Ocular count of whole sample, or of a known portion (aphid counting grids).

- B. Weight or volume measurement, with conversion factor.

In the sampling of soil fauna the best progress toward rendering all three of these steps really mechanical has been made, and in the sampling of foliage insects the least. Yet most of the long-term studies on population dynamics are concerned with foliage insects. The importance of developing improved methods for the extraction of insects from foliage and the effect which such methods could have on design and cost have been demonstrated (67).

As Finney (34) has pointed out, the mechanics of sampling nearly always give rise to certain errors, the magnitude of which determines the accuracy of the counts. (Precision, on the other hand, depends on statistical sampling error as determined by population variation and sample size.) Few, if any, of the extraction methods are fully efficient, but in the case of purely mechanical methods a constant correction factor can usually be determined and applied. In the case of hand-picking, variations between individual workers and between the same workers at different insect densities make extensive checking necessary (67, 74). Some biologists believe it is imperative to aim at 100 per cent accuracy, so that the only variation arises through sampling error. Theoretically this is so, but the law of diminishing returns becomes important as accuracy approaches 100 per cent, and it must often be decided whether available sampling time should be spent, say, in counting 100 sample units with 95 per cent accuracy, or only 20 units with 98 per cent accuracy. There is usually a relatively large variation between sample units in natural populations, and it is questionable whether much is gained by striving for a degree of accuracy that is out of all proportion to the precision with which population parameters can be estimated from the sample.

DISTRIBUTION

Population distribution is another factor that affects the design of sampling and, like mechanics, represents an aspect of sampling that would require by itself the full space of this review for adequate treatment. It is becoming increasingly evident that insects (as well as the plants or other insects on which they feed) are seldom distributed randomly in nature, and that this poses important and often difficult questions concerning both design and analysis. It is possible here only to define the major aspects of this problem and to suggest some leads to the literature.

Frequency distribution of population data.—Once some preliminary sampling has been done, it is relatively easy to test the frequency distribution mathematically to learn whether it is random, "over-dispersed" (suggesting

clumping or aggregation), or "under-dispersed" (suggesting more uniform spacing than would be expected from random dispersion). One large sample can be used in a direct test between actual and expected frequencies, or a number of smaller samples which show the relation between mean and variance. If the test indicates anormality, the problem of skewness can be bypassed by using a more appropriate measure of central tendency, such as the median or the mode. It is generally more useful, however, to transform the original data to another scale so that the usual statistical procedures can be employed. The transformed data are then tested to ensure that the transformation satisfies the criteria of additivity and homogeneity (13). If the objects of the sampling are concerned with general survey, or with analyses of variance between different populations or treatments, this may be as far as the entomologist needs to go. If they are concerned with more basic population research, he may want to go farther (see below). Appropriate tests for randomness and suggested transformations are given in most of the statistical handbooks and in some of the textbooks cited earlier (2, 72), but the subject is treated in better detail by Bliss & Calhoun (13) and Quenouille (75). A point often overlooked is that at least two parameters are estimated by each sample—mean and variance. Effective transformation permits the pooling of variance estimates and thus increases the sensitivity and significance of the data.

Spatial distribution of population.—The frequency distribution of sampling data may or may not reveal the true distribution pattern of the population, because the former has two components—a biological one that reflects the spatial distribution of the animals and a statistical one that reflects only the nature and size of the sample unit. There is adequate evidence from entomological sampling that the nature, size, and arrangement of sample units can affect both the type of frequency distribution indicated by the data and the relative degree of skewness (14, 62, 103). In detailed work on population dynamics, it is often desirable to separate these two components because the spatial distribution of the population, and the behavioral or other characteristics that determine it, may be important factors affecting such things as the searching of parasites or the spatial relation of mortality to population density. The separation of these two major components, and the elucidation of the several factors that are no doubt usually involved within the biological component, should be a very fruitful field of research for entomologists who are mathematically inclined. Cole (22) pointed this out some years ago, and pertinent literature has recently been reviewed by Torri (96) and Waters (103), who conclude that much remains to be done. Distributional studies in phytosociology (23) and other fields of ecology (20, 84) will also contain much of interest to the entomologist.

Most insect populations appear to be overdispersed, for they frequently approximate the negative binomial distribution (14, 67, 103), or one of the contagious distributions (9). Work by Bliss and associates (12 to 15), Beall (8, 9), Skellam (84), Anscombe (3) and Waters (103) deals with such distri-

butions. These authors have demonstrated that several different biological assumptions can lead to the same sort of frequency distribution and that caution must therefore be exercised in drawing biological inferences from sampling data alone. Also, at low population densities these distributions often approach the Poisson, so it cannot be assumed that dispersion is necessarily random if the data were obtained only at low densities (84). On the other hand, the appearance of contagion may result from intermingled random distributions (22).

Temporal distribution of population.—The distribution of animal populations in time has not yet received as much attention as their spatial distribution. The analysis suggested by Torri (96) will be of interest to entomologists, and more particularly the comparisons by Leslie (58) and Skellam (85) of stochastic and deterministic models for temporal analysis. In view of the fact that insect population changes in time are geometric rather than arithmetic, transformation to logarithms often facilitates analysis and interpretation (68). The discussion by Stevens of different scales of measurement and their interpretation is pertinent in this regard (92). If the object of a long-term study is to construct a mathematical population model, the entomologist may be more concerned with the frequency distribution of survival or mortality ratios than the distribution of population estimates, because these ratios appear to be a logical expression of the dependent variable (rate of population change) in either empirical or deductive models.

Nearly all the authors cited in this section have emphasized the need for more research before the numerous questions posed by distributional features can be answered. The entomologist is often at a loss to know when it is safe to use sampling data in their original form and when he must transform, and often the statistician cannot advise him. He is on more familiar ground when working with untransformed data, but faces the possibility of reaching erroneous conclusions [cf., Finney & Varley (36)]. More work is badly needed like that of Finney (33), in which the efficiency of estimates expressed on original and transformed scales is compared and methods for re-transforming the mean and variance back to the original scale are suggested.

DESIGN

If sampling is a mixture of art, science, and drudgery, then the art and science are mainly in design. However, with the development of mathematical procedures for the optimum allocation of sampling resources, the art required in earlier years has been largely removed. It is helpful, of course, if the investigator knows in advance where the major sources of variance are likely to lie, for this will help him to design the preliminary sampling with skill. Once the preliminary data are on hand, however, all questions concerning the final, optimum design can be answered mathematically. For the methods of doing this, textbooks devoted to sampling (21, 47, 81) should be consulted rather than the short sections on sampling in general statistical handbooks.

There are three main aspects to design: selection of the universe, which depends directly on the object of the study, selection of the sample unit, and determination of the optimum stratification and distribution of units within the selected universe. A fourth aspect discussed by Finney (35)—selection of the unit of measurement—usually resolves itself to insect counting in direct sampling but sometimes poses problems when such indices as frass measurement are used (65). In the selection of the sample unit there is a tendency to select too large a unit for the preliminary sampling, with the result that it is not possible to test the relative efficiency of smaller units. In making a design for foliage insects, for instance, the unit for preliminary sampling should preferably be as small as a leaf or a shoot, and estimates of variance should be obtained within branches, between branches in different vertical and horizontal portions of the plant, and between plants. If the analysis of the preliminary sample shows that a whole branch would be a more efficient unit, it is then easy to make the change. Most insect sampling has suggested, however, that small units, carefully stratified, yield the most efficient designs. Examples of preliminary intraplant work of this nature are presented by Bradley (16) for potato aphids and Church & Strickland (19) for cabbage aphids. Similar examples for forest insects, illustrating the use of variance components in determining optimum size of unit, are presented by Ives (51), Morris & Reeks (69), and Stark (91). Another important value of the small unit is that it permits better separation of the biological and statistical components of skewness (103).

Estimating total population over large areas.—It appears there is rarely any need to estimate total or mean insect population over very large areas, and in some cases where it has been attempted the author has not specified why the estimate was required or whether any use was made of it. However, there may be some merit in estimating total locust population on certain breeding areas, so that swarming and hazard can be predicted (80), or in estimating insect population over a large political area as an index of economic damage, provided the insect population is easier to sample than the degree of damage itself (94). The design of such surveys is very similar to the design of a human census, employing stratification to great advantage and observing, of course, the principle of randomness. Examples are described by Beall (7) for potato beetles, Bancroft & Brindley (5) for corn borers, and Kaelin & Auer (54) for a forest defoliator. It is interesting to note that all three of these examples have senior authors whose interests are primarily statistical, that all three illustrate sophisticated stratification and optimum design in respect to area, but that the choice of the smallest sample unit was made in some cases more by arbitrary than optimum procedures.

Mapping population over large areas.—The objects of studying insect populations over very large areas are more often concerned with mapping population or infestation classes than with estimating total or mean population for the area. This is easier and more economical because sequential sampling can be used and because there is no need to randomize the location

of sampling points. Thus, in both the continuous forest insect survey across Canada (61) and in special surveys of a province or more to assess the need for chemical control (106), the location of sampling points is largely a matter of accessibility. Even along accessible roads, trails, or waterways the selection is more often systematic (intervals of one mile or so) than random. The sampling data are often plotted on a map as the survey progresses, so that special efforts can be made to fill in any large blank areas.

As DeLury (27) has pointed out, the term "random sample" is often misused by biologists: "... in certain circles, there cannot be such a thing as a plain sample; it must be a 'randomsample.' Like 'damyankee,' it is all one word." Very little insect sampling is truly random. In some cases we do not know how to randomize, especially for motile species, and when we do know, the cost function shows that it is too expensive for most purposes. Thus, systematic sampling, cluster sampling, and even very purposeful sampling, are often called random, whereas at best they might be called unbiased. In the extensive sampling by Webb (106), for instance, there is presumably no relation between budworm population and the location of roads and trails, so he could use his points for a given area to estimate mean population or mean class. In theory, at least, he could not estimate variance and confidence intervals without fear of criticism because all statistical procedures are based on a random distribution of sample units. In practice, however, biologists very frequently apply these procedures to any type of unbiased or systematic sampling, and it appears to be still uncertain, from statistical literature, whether this represents a serious violation or not. The question is discussed by Gulland (45), Schumacher & Chapman (81), Snedecor (87), and others. The main point to bear in mind is that most of the objectives of insect sampling do not require strict randomization, at least in regard to area, and the additional expense that it imposes is therefore not always justified.

Relating population to edaphic factors.—It is becoming increasingly evident from field data that fluctuations in insect population take place at different average densities in different environments, and that outbreaks may be encouraged by certain edaphic factors. The elucidation of these relationships, using population density as the dependent variable and such factors as topography, soil type, density and distribution of host plants as the independent variables, often calls for a design somewhere between extensive survey designs and intensive population dynamics designs. Only two examples will be discussed.

Kaelin & Auer (54) had the main objectives of (a) estimating the mean larval population of a forest defoliator [*Eucosma griseana* (Hübner)] in the Engadine Watershed of Switzerland and (b) finding how population was related to edaphic factors. To satisfy the first objective, they had to apply random sampling within the Watershed and to satisfy the second objective, they stratified as follows: Engadine Watershed → 11 human communities (for convenience in collection of samples, which was done by local inhabi-

tants) \rightarrow 34 partial areas (each homogeneous with respect to altitude and direction of slope) \rightarrow 1234 sections (each homogeneous with respect to altitude, slope, and vegetation). The particular sections to be sampled each year were chosen in advance by use of random numbers and within each section a cluster of trees was located randomly on a map of the section. Sample branches were collected from a number of trees (generally three) in each cluster, and the only departure from randomization in the whole design was the attention given to the ease of climbing in selecting individual trees. Optimum allocation procedures were used to modify the design and calculate required sample size each year. Weighting was on the basis of both size and variance, and partial regression methods were used very effectively to elucidate the effects and the interactions of the independent variables.

Morris and associates (67, 70) had the main objectives of (a) learning how spruce budworm populations were related to edaphic factors on the Green River Watershed of Eastern Canada and (b) elucidating the mechanism through which these factors and other factors determine budworm numbers. Since the estimation of total population on the Watershed was not an object, a nonrandom design was permissible. Defoliation and tree mortality were used as indices of population and large numbers of small homogeneous plots were established in different types of forest stands, in which some 15 independent variables were recognized and measured. The location of these plots was purposeful, with a view to sampling as many different conditions and combinations of conditions as possible, and a deliberate effort was made to make the design reasonably orthogonal. The analysis of the effects of edaphic factors was again made by partial regression methods and will shortly be described by D. G. Mott. Within this broad structure, the principle of double sampling was used to elucidate mechanisms and to study other factors. That is, a small number of the plots, selected very purposefully to represent widely different conditions, was used for detailed sampling of the insect itself and the development of life tables in successive generations. The principle of double sampling can be valuable in entomological work and other examples of its use are presented by Banks (6) and Wadley (100). Within the special plots used for life table work, clusters of trees were selected for study, largely on the basis of ease of access, with stratification on the basis of species, relative height, and flowering condition. Numbers of branches per tree, trees per cluster, and clusters per plot were determined by optimum allocation techniques. Cluster sampling, as opposed to unrestricted random sampling, is another device used frequently in modern sample design to reduce the cost of movement between sample units (47).

Examples of alternative designs could be cited, but the contrast in design between these two projects will serve to illustrate the dependence of design upon objectives. Stratification is common to most designs and the greater the population difference between strata, the greater the gain in efficiency

through stratifying. This is well illustrated in a series of papers on sampling designs for sugarcane insects (56).

Studying population dynamics.—The objects of sampling in detailed studies on population dynamics are generally two: (a) to estimate mean density in a small area or restricted universe, because population trends are needed and because density is usually an important variable affecting survival, and (b) to estimate survival (or mortality) not only for each successive generation of the insect but also for shorter age intervals (stages, or even larval instars) within the generation. Selection of the study plots is usually very purposeful, rather than random, and there is often severe universe restriction within the plots. Thus Prebble (74) found variance too high when he attempted to sample sawfly cocoons on a plot without respect to tree positions and consequently restricted his universe to the area directly under the crowns of average co-dominant trees. Under each crown he used four, 1 sq. ft. quadrats. Ohnesorge (73) used even greater restriction for cocoon sampling by taking all samples from the same side of the tree. Varley (99) used quadrats 1 sq. meter in size and, to reduce variance, restricted his universe to areas that contained "a fair quantity" of knapweed, which was patchy in distribution. Universe restriction is a sensible device in such studies, although it may be pointed out that all these workers might have benefited by using optimum rather than arbitrary procedures in determining both the size of the sample unit and the number and distribution of units within the restricted universes.

When the objectives require periodic sampling in the same population, it is also helpful to use a design that permits successive samples to be analyzed like "paired variates." In forest insect sampling, for example, the major source of variance is often intertree rather than intratree and successive samples are often correlated. That is, the individual trees in a cluster that harbor the highest insect population in one generation are likely to do so again the next generation. Different trees of the same species, or even branches from different parts of the same tree, are not equally susceptible to insect attack, and this is one of the biological components of nonrandom distributions. If the same trees are used for successive sampling, the sensitivity of temporal population comparisons is increased in proportion to the correlation coefficient (67, 74). Geier (41) has demonstrated similar correlations for insects attacking fruit trees. This principle is carried to its ultimate in the study of sessile forms, where the unit of substratum is permanently marked and successive counts are made on the same unit (38).

SAMPLE SIZE

In the last section reference was made to various textbooks and to a few entomological examples illustrating the calculation of optimum sample size, and it was indicated that this should be based on variance components and cost function formulae, the latter being generally expressed in terms of man-

days. It is surprising that there appear to be less than half a dozen entomological papers in which any cost function has been derived. If he did not know better, a reviewer might conclude that the resources for most insect sampling projects are so unlimited that this is not a pertinent consideration!

Sample size may be calculated as the required allocation for a specified degree of precision or the optimum allocation for the resources at hand. The latter is often more useful for two reasons—(a) resources are nearly always limited and (b) for most entomological purposes it is not yet known what precision is needed. Extensive sampling is generally conducted for economic purposes, especially for assessing the need for direct control, and it is not difficult to assign an acceptable degree of precision depending upon the economic consequences of being wrong. In sequential sampling, for instance, a risk of classifying one point in ten incorrectly is often accepted because the results are plotted on a map and the scattered aberrant points are not likely to lead to improper classification of any large areas. In intensive sampling, on the other hand, our idea of required precision is extremely vague because no detailed field projects on population dynamics have yet been satisfactorily completed and analyzed. In other words, we do not know yet what proportion of the variance in survival or mortality it may be possible to explain with a given degree of sampling precision. We become disturbed if our standard errors very much exceed 10 per cent of the mean. Yet who can say that we may not be better off, in the final regression and structuring, if we accept higher errors and use the extra time to study several times the number of plots, especially if this provides a much wider range of population densities or environmental conditions?

Milne (64) pointed out the frequent inadequacy of insect sampling and referred to some of Varley's (99) data as follows:

Now an estimate such as a mean of 6.9 ± 1.6 from a sample of 10 sq. m. (p. 146) signifies no more than this: there is a strong chance (19 to 1) that the true mean of the population does not lie outside the limits 3.3 and 10.5 per sq. m., i.e., that the population mean is somewhere—*anywhere*—between 3.3 and 10.5.

This is true, although the probability is higher, of course, that the population mean will be closer to the estimated mean than to one of the fiducial limits. However, we are not likely to learn what precision is required by such pessimistic contemplation of individual standard errors. Experiments on population dynamics in controlled environments are often criticized if they do not extend over a considerable number of population cycles, and by the same token field studies should not be expected to produce very strong conclusions if they are not continued over a reasonable period of years. By such extension, as well as by replication each year in different environments, a large series of population or survival estimates can be obtained along with corresponding estimates of the main independent variables. Thus, even though the standard error of any single estimate is fairly large, regressions based on large numbers of such estimates may still show significantly what

factors determine survival and how they interact. The analysis of adequate long-term data will be concerned more with population trends and survival regressions than with the statistical comparison of two individual densities, and Varley's degree of precision might have been adequate for his purposes if his work had extended over a longer period than two years.

At the same time, some workers seem to have been overly impressed by the similarity in population trend between different sample units. DeBach (25), for example, suggests that the study of one tree in a citrus grove may be sufficient because the population changes on one tree will reflect adequately the population changes in the whole grove. In working with such a small sample, or even with the larger clusters or plots more commonly used in detailed population studies, true survival trends can easily be confounded by the migrational movements or random aerial dispersals to which many insects are subject. If such movements are random, it is easy to show that initially low population trees or plots will lose relatively less insects during such periods than initially high ones. In this regard Dempster (28) describes a technique developed by Skellam for separating the effects of mortality and movement when locust populations are studied on adjacent plots. Even very large plots would not be free of such influence during moth flights of the spruce budworm, and methods have been worked out for estimating the degree of immigration or emigration (70).

DISCUSSION

Extensive sampling.—There have, perhaps, been a few instances of extensive sampling in which the elegance of modern design appears to have seduced the entomologist into estimating parameters of limited biological significance. More often, however, the opposite has been true and full advantage has not been taken of the effective statistical tools that now exist for designing extensive surveys. This applies particularly to such techniques as sequential sampling and to the use of optimum allocation procedures and cost functions. It has also been suggested here that for extensive coverage, or even for limited coverage when the objectives require only one population estimate in each generation of the insect, it may often be worth while to consider various types of indices before embarking upon the time-consuming practice of direct counting. The effective use of population indices in fish and wildlife work, and particularly the use of regression methods for converting raw indices to useful measures of relative or absolute density, should provide valuable leads.

Intensive sampling.—In intensive sampling, particularly for population dynamics research, the situation is much different and there is very little literature on which to base a discussion. This type of sampling is still in its infancy and all the necessary statistical tools do not yet exist. This applies particularly to procedures for separating the biological and statistical components of aggregated distributions, using transformations effectively, and compensating for distributional features both in design and in analysis. In

fact, the entomologist does not even know what degree of precision he needs in such sampling and the statistician cannot help him, of course, until some of the existing field projects have achieved sufficient temporal and spacial replication to permit an adequate analysis. Again, developments in stochastic procedures are relatively new and it is too early to foresee their possible effects on either design or analysis.

It must be admitted that less progress has been made in entomology than in fisheries research towards bridging the gulf between population theory and field data. The analyses and mathematical models described by Beverton & Holt (11) and Ricker (78) have as yet no effective counterpart in entomology. This results partly from inadequacies in design, partly from the fact that intensive field studies are not continued through enough generations of the insect, and partly from the reluctance or inability of entomologists to attempt adequate analysis and interpretation of their data. Varley (99) made a very worth-while contribution when he demonstrated that the terms in a deductive insect model are not pure abstractions but are susceptible to measurement and testing in the field. Analyses of this type, whether based on adequate or inadequate data, are more refreshing than the long tabulations or histograms in which undigested data from entomological field studies are often presented. It does seem an anticlimax, after the trouble and expense of developing elaborate sampling methods, if the results are not fitted at least to an empirical polynomial by partial regression methods, and this will lead almost inevitably to the development of joint deductive-inductive models as the entomologist tries to gain an insight into the processes that determine insect numbers [Watt (104)]. The object of such models is not so much to predict natural population changes, because some of the independent variables themselves cannot be predicted, but more to suggest methods of minimizing insect populations (as compared to maximizing the yield of fish, game, or crops) so that biological, silvicultural, and chemical control measures can be elevated from their present empirical position.

If this sounds too idealistic, it may be of interest to consider the following comparison by Elton & Miller (31):

The early organic chemist must have felt very much as the animal ecologist often does now. It must have seemed dangerous and vain to look beyond, say formaldehyde or a benzene ring, towards higher complexities. Yet organic chemists now seem to move with great confidence amidst compounds of complicated structure, built together by steady research upon related chemical compounds and on the general properties of such systems. Perhaps it is fairer to assess the present position of the animal ecologist as comparable with that of the *inorganic* chemist at the time of Dalton!

Finally, the most basic point of all concerning intensive sampling is that we do not yet know whether it will be an effective tool for disclosing the mechanisms of population regulation. It is noteworthy that most of the population theorists have chosen to use controlled laboratory populations and that many field workers have become discouraged by the magnitude of sampling error, sampling difficulties when populations are very low, or the

great complexity of factors affecting natural populations. On the other hand, there is the dedicated field man who stubbornly refuses to accept any relationships demonstrated in a controlled and confined environment. It is becoming evident, however, that both approaches are useful and that each has a great deal to contribute to the other. Questions concerning the relative efficiency or practicability of the field sampling approach can only be asked in this review, not answered. Some preliminary encouragement is offered by the excellent fit of Miller's (63) field data to Watt's (105) model for parasite attack, and by the significance of prediction equations based on a single independent factor (68). In both these studies the standard errors of the population data were variable but might average 15 per cent or more. There are seven field projects known to me (and no doubt others exist) devoted to the long-term study of the population dynamics of a pest insect and its principal enemies. After some of them have reached the stage for adequate analysis, a future reviewer may be able to say whether or not the approach is successful and to suggest the direction in which future intensive sampling should be directed.

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THE USE OF HONEY BEES IN THE PRODUCTION OF CROPS¹

BY FRANK E. TODD AND S. E. MCGREGOR

Entomology Research Division, Agricultural Research Service,
United States Department of Agriculture²

The use of the honey bee as a tool in the production of agricultural crops is a comparatively recent development. Waite's 1894 publication (66) on "Pollination of Pear Flowers" stimulated great activity in the study of fruit pollination. During the early part of this century, horticulturists became aware of the magnitude of the need for cross-pollination among fruit varieties and of the economic necessity for insect activity to cross-pollinate them [Dorsey (14)]. Horticulturists stressed the importance of the honey bee as a pollinator of fruit blossoms and contributed much information on the behavior of bees in the orchard. To provide for successful cross-pollination, plans for interplanting of varieties had to be based on knowledge of bee activity in the orchard. Apparently the renting of colonies for orchard-pollination service had its beginnings around 1910. The practice grew rapidly during the next score of years. Today the honey bee is recognized in most of the world's agricultural areas as the most important pollinating insect.

The demand for pollination service stimulated several apiculturists to study bee activity in orchards during the 1920 to 1940 period. Their studies contributed substantially to our understanding of effective methods of using bees for pollination. The acute demand for legume seed during World War II promoted research into the value of bees in the production of seeds. During the past 20 years, the use of honey bees for the production of legume seeds has been the center of bee-research interest. While no statistics are available on the extent of bee-rental traffic, possibly a million colonies are rented annually for pollination service in the United States. However, bees are rented only where the pollination problem is acute; a far greater pollination service is provided free of charge by the other portion of the bee industry. In fact, the bee industry's chief importance to the nation's economy is the pollination service it maintains for agriculture.

Our common honey bee (*Apis mellifera* Linnaeus) was indigenous to Europe and Africa where it has been maintained in hives for many centuries. Within historic times, it has spread with the aid of man over most of the world. A world-wide beekeeping industry developed because of the honey bee's ability to survive and store large surpluses of honey under a wide variety of climatic conditions.

Our knowledge of the other three species of honey bees (*Apis indica* Fabricius, *Apis dorsata* Fabricius, *Apis florea* Fabricius) has been advanced

¹ The survey of the literature pertaining to this review was completed in August, 1959.

² In co-operation with the Arizona Agricultural Experiment Station.

by the recent publications of Butler (8), Muttoo (43), and Lindauer (38). These species are all indigenous to south Asia, China, Japan, and the Philippines.

The eastern honey bee (*A. indica*), although different in many respects from our common western honey bee (*A. mellifera*), constructs a similar nest in a cave or other cavity by suspending several parallel beeswax combs from the ceiling. This species has been maintained in hives and is the basis for the apiculture of India, China, and Japan.

The giant (*A. dorsata*) and the dwarf (*A. florea*) honey bees have not been successfully maintained in hives. Their nests are always constructed in the open by suspending a single beeswax comb from a ledge or branch of a tree or shrub. Living in the tropics usually makes storage of large honey surpluses unnecessary for survival. They habitually abscond from their nests when local conditions become unfavorable.

Butler (8) suggested that the eastern and western honey-bee species arose from a common ancestor in south Asia during recent geologic times. The eastern species retained the more primitive habits and spread over tropical and sub-tropical Asia, while the habits of the western honey bee became modified for survival in the temperate and subarctic climates of Europe as well.

NUMBER OF COLONIES PER ACRE

In 1916, the horticulturist Hendrickson suggested colonies be obtained from beekeepers and scattered in prune orchards during the blooming time at the rate of one colony per acre and then removed (27). Although the use of bees in the pollination of many crops has been studied, it has become apparent that the principles of using bees in orchard pollination apply equally to field and vegetable crops. The term "one colony per acre" has become a fixture in pollination literature, not only for fruits [Griggs (23); Stephen (58)], but for melons [McGregor & Todd (39); Pew *et al.* (51)] and many of the legumes [Todd & Vansell (60)]. However, more colonies per acre are recommended for such profusely flowering plants as alfalfa and red clover. Recent apicultural research has been concerned with problems which limit or increase the effectiveness of colonies used for pollination service.

STRENGTH OF COLONIES

Apiculturists are aware that the colony is not a standard unit and that the term "one colony," as used in literature, needs to be defined. This problem has been studied by Hutson (29), Filmer (18), Farrar (16), Woodrow (70), Brittain (6), Vansell (64), and Gooderham (22). These investigators are unanimously agreed upon the superiority of "strong colonies" for pollination service. Their studies show that a greater percentage of bees fly from strong colonies than from weaker colonies and that flight begins at lower temperatures. Brittain (6) showed that, while there was some flight as low as

11°C., flight activity stepped up at 16°C. and showed a steady increase up to 29°C. Low temperature flights are of interest to fruit growers who require pollination in the spring when the weather is cold and stormy.

The magnitude of flight activity and pollen collection by the colony has been correlated with the amount of brood rearing. According to Lindauer (36, 37), all field activity and the division of labor of the field force (nectar, pollen, and water gathering) are regulated by the needs of the colony. For example, if the colony has a large quantity of brood to feed and is short of pollen, more of the field force will gather pollen. If temperatures become high and there is need for water to cool the hive, more bees will engage in collecting water. Lindauer (36) compared colonies with and without pollen traps and found a larger percentage of pollen gatherers in the colonies with pollen traps. It has been suggested that pollen traps could be used to stimulate pollen collection, but, in our own experience, colonies tend to adjust their brood rearing to the available supply of pollen in the hive; therefore, the use of traps for this purpose would probably be a practice of questionable value.

Strong colonies are usually recommended in pollination literature, but attempts to define this term vary widely. Such terms as "thousands of bees," "frames of brood," "square inches of brood," "the number of frames the cluster covers," and combinations of these are commonly used. Generally two factors require definition: bee population and the amount of brood. The data in Nolan's studies of brood-rearing cycles (45) show that his best colony at maximum development had approximately 1200 sq. in. of brood (all stages) distributed over 12 combs. However, it had maintained an average of about 900 sq. in. during the main portion of the active season. This colony was considered better than the average. Similar data (unpublished) gathered from 100 colonies in California show that they averaged eight frames of about 100 sq. in. each per colony.

A precise estimate of the bee population of a colony can be made by weighing the bees after shaking them from the combs [Farrar (17)]. Experienced apiculturists can make a fairly good estimate of colony strength by counting the combs covered by the cluster. However, time of day, temperature, and amount of field activity must be considered in this estimate.

A California beekeepers' cooperative, which contracts pollination service for alfalfa pollination, guarantees an average of 800 sq. in. of brood per colony [Todd (59)]. This amount of brood amply covered with bees in a two-story colony appears to be a practical definition of a satisfactory colony for pollination.

Jeffree (30) selected and photographed eight combs with bees on them, then counted all the bees. The numbers ranged from 300 up to 3000. The latter number was all the bees that could cluster on one comb. The combs he used were about one-fourth smaller than the standard size used in the United States. By comparing the cluster of bees on each comb to similar clusters in

the photographs, the beekeeper may obtain a fairly accurate estimate of the colony population.

DIRECTING BEES TO SELECTED CROPS

Directing bees to specific crops has received considerable attention because bee activity cannot be confined to a selected field or orchard. Gubin (25, 26) and von Frisch (19) investigated the principle of feeding a colony with sugar syrup scented or flavored with flower extracts from the target source or by garlanding the feeder with flowers from a desired source. They and several others reported increasing visitation, honey production, and seed yields by use of these methods. These experiments are extremely interesting, but, as pointed out by Ribbands (55), the technique has not come into general field practice. It seems likely that scattering colonies within the crop to be pollinated may take better advantage of the scent principle of directing bees. At any rate, placing colonies in the field has given satisfactory results in practice. For example, Vansell (65) found that the alfalfa-seed yield within 100 yards of colonies was 30 per cent higher than that 300 yards away, indicating a concentration of bee activity close to colonies placed in the field.

Von Frisch (19) pointed out that bees will not continue to visit a crop which yields nothing. Therefore, perhaps more progress could be expected from improving the attractiveness of a crop than from trying to direct flight. Pedersen (49) found a significant correlation between nectar sugar per plant and honey-bee visitors and seed yields. He also found a wide range in the amount of nectar sugar produced in a group of alfalfa plants and a heritability of more than 60 per cent was indicated. Pedersen *et al.* (50) indicated increased nectar production and improved seed production by row spacing and thinning alfalfa. There appears to be great opportunity for improving pollination by both plant breeding and cultural practices.

PLACEMENT PATTERNS

Patterns of placing colonies in orchards or seed fields have received considerable attention. Hutson (28) and Murneek (42) found that the number of visitors to fruit blossoms decreased with distance from the hives and suggested that single colonies be placed at 210-foot intervals in the orchard. Philp & Vansell (52) recommended placing groups of 10 to 20 colonies at approximately the same rate. In extensive studies by Brittain (6), counts of bee visitors to apple blossoms declined beyond 200 yards and few visitors were found beyond a quarter of a mile from the colonies. He recommended that groups of colonies be placed at 440-yard intervals. Butler (10) suggested that the groups be placed in the center of 15 to 20 acres of orchard or at intervals of 176 yards in larger orchards.

Vansell's observations on seed set in alfalfa near the hives have already been cited (65). Walstrom *et al.* (67), working with red clover, found a dis-

tinct break in seed yields between 100 and 200 yards from colonies. Studies at the United States Department of Agriculture Bee Culture Laboratory at Tucson, Arizona, showed no significant difference in the percentage of alfalfa pod-set up to 100 yards from the colonies, but the pod-set dropped from an average of 55 per cent within the first 100 yards to 42 per cent at 150 yards. Seed yields of five sampled fields followed the same trend.

There is evidence that the most effective pollination activity occurs within a radius of 100 yards from the colonies. Therefore, the colonies should be located at intervals which will overlap at 100 yards. Six and one-half acres would be included in the 100-yard circle, thus indicating that colonies may be placed in groups rather than singly. The number of colonies required per acre would indicate the size of the groupings; for example, seven in a group would provide one colony per acre, while 13 would provide two, and 20, three per acre.

Placing a single large apiary on the edge of the field or orchard is an inefficient use of pollinators. Better service can be expected if the colonies are placed within the field or orchard. Sorensen *et al.* (57) suggested a pattern with heavy concentration of colonies in the center of the field and smaller groups around the borders. In practice, the California pollination cooperative places groups of 10 to 12 colonies in rows 528 feet apart each way, the outside rows being 264 feet inside the borders of the alfalfa field.

FORAGING AREAS OF INDIVIDUAL BEES

Foraging areas of individual bees have been investigated [Minderhoud (41); Buzzard (11); Crane & Mather (13); Butler *et al.* (9); Ribbands (54); Singh (56)]. Individual foraging bees do not wander at random but limit their visits to comparatively small areas of 10- to 20-foot circles. They are constant to a single species of plants and fairly constant to the foraging area. However, when the nectar source begins to fail, they may extend their working area and some individuals may begin to wander.

Ribbands (55) found larger foraging areas in the absence of competition between bees and smaller areas associated with intense competition. Competition should result in more thorough working of all the flowers in the foraging area. He concluded that, while competition decreases the foraging area, it also causes more rapid cessation of flowering. Obtainment of increased production of many crops through more thorough cross-pollination is associated with the activity of individual bees and should receive more attention.

POLLEN DISPENSING

Burrell & King (7) developed a device for dispensing hand-collected pollen on the bees leaving the hive so they carry the pollen to the field and cross-pollinate orchard trees. Modifications of this device, usually called a pollen insert, have been made by Kremer (34), Karmo & Vickery (32), and Townsend *et al.* (63). In use, hand-collected pollen from a desired source is mixed

with lycopodium spores and placed in the pollen inserts. Bee traffic out of the hive is diverted so that the bees must walk through the pollen. Inconclusive results or failure to get a satisfactory set of fruit by use of the pollen insert were reported by King & Burrell (33), Overley & O'Neill (46), Webster *et al.* (69), and Griggs *et al.* (24). However, Karmo & Vickery (32) and Townsend *et al.* (63) have reported satisfactory fruit set by the use of pollen inserts. Although somewhat expensive, the pollen-insert method is occasionally used to provide the proper pollen for effective cross-pollination of such high-priced, short-flowering crops as plums, apples, pears, cherries, and almonds.

MOVING COLONIES

The custom of moving bees to different areas for honey production is at least 4000 years old. The Egyptians operated their bees on barges moved up or down the Nile. In modern times, beekeepers have moved their bees by boat, wagon, train, and motor trucks to new or better pasture. Under any condition, moving bees is hard work, much of which must be done at night when the bees are in the hives and by operators accustomed to bee stings.

The use of bees in pollination of agricultural crops requires movement of large numbers of colonies within short periods of time. To comply with this demand, the industry has developed mechanical loaders. Two general types of loaders are now in use: (a) the boom-type, which has a mechanically operated lifting device on the end of a pivoting boom for single colonies, and (b) a modified tractor fork-lift, which handles groups of colonies on pallets. With either of these, one man can load 100 or more colonies on a large truck at nightfall and, before daybreak, distribute them in another field many miles away. The development of loading devices has contributed greatly to the efficient use of bees in large-scale crop pollination.

WATER AND TEMPERATURE

Beekeepers generally recognize that colonies require large quantities of water, but the importance of providing water for bees in colony management is frequently overlooked. Water is used to dilute brood food, for adult use, and for cooling the cluster. Lindauer (37) has recently investigated the water economy of the colony. He estimated the labor of one water carrier for an entire day was required to supply water for 20 larvae. Some of the water is obtained from incoming nectar, but he observed that bees carried water every day of the active season. The number of water carriers increases with the colony's need for water. When outside temperatures reach 30°C., bees begin preparations for cooling the hive. They spread apart on the combs, and, as the temperature rises, they begin fanning at the entrance and cluster on the outside of the hive. They spread water in a thin film in uncapped cells. They place droplets in depressions in the cappings and on top of the combs. The droplets are also hung from the upper wall of open brood cells. Bees on

the combs fold and unfold their proboscises, exposing a film of water between the proboscis and the lower part of the mouth. The fanning by the bees creates a circulation of air over this water, and its evaporation cools the hive.

In southern Italy, Lindauer (37) compared the number of nectar and water carriers entering two shaded colonies with the number entering one colony exposed to the sun. In the early morning bees from both were carrying nectar, but after the temperature reached about 30°C. nearly all of the field force of the colony in the sun became water carriers though nectar collecting continued in the shaded colonies. This means that the colony's need for water must be very exacting, and apparently bees will abandon the gathering of foods from flowers when the demand for water becomes acute. Water deficiency may seriously impair the colony's efficiency for pollination or honey production.

Owens (47) shows that critically high temperatures can be modified by shading the colonies from direct rays of the sun and heat radiation and by use of heat-reflecting paint. Maximum protection is afforded when they are placed on green vegetation and in dense shade of trees. This alleviates, but does not eliminate, the need for water. From a practical standpoint, therefore, water should be readily accessible to colonies used in pollination service.

DRIFTING

When colonies are moved into seed fields, the foragers sometimes become confused when making their first flights and settle in large numbers on a few hives. This depletes the field force of the abandoned colonies and impairs the working efficiency of the colonies receiving the drift. No studies that duplicate this type of drifting have been made.

Free (21) moved single hives different distances up to one mile, and 87 per cent of the marked bees returned to the new site. When he moved them five miles away, 94 per cent of the marked bees returned to the new site. Nekrasov (44) found that 10 per cent of the bees drifted into colonies in the three center rows of a ten-row bee yard during their reorientation after removal from winter quarters. During reorientation, immediately after an apiary is moved, the bees may drift in unexpected patterns.

In established apiaries, a different type of drift may occur. Nekrasov (44) showed that, during the honey flow, the bees drifted into colonies nearest the direct line of flight from the nectar source. He studied the honey production of six apiaries with over 200 colonies each, dividing each into four blocks for record taking. He found the range in production between the best and the worst blocks in these apiaries varied from 6 to 51 per cent, which he attributed to drift of foragers between blocks. When Nekrasov studied rows of hives instead of blocks he found that the mean yield of each apiary decreased, row by row, as the distance from the nectar source increased.

Free (20) reported that drift between colonies varied with conditions. When hives were arranged in repetitive patterns, marked bees drifted to hives in similar positions in other rows; arranged in single rows they drifted to the end colonies. Drifting was considerably reduced by facing colonies in different directions and painting the hives different colors.

BEE POISONING

When colonies are distributed in fields or orchards, the use of insecticides may become an acute problem [Todd & McGregor (62)]. This problem has been attacked in both the laboratory and field. Laboratory tests with caged bees have provided data on the median lethal doses (MLD) and the relative toxicity to individual honey bees of most of the currently used insecticides [Anderson & Tuft (2); Atkins & Anderson (3); Büttcher (5); Eckert & Tucker (15); Palmer-Jones (48); Postner (53); Weaver (68); Woodrow (71)]. Unfortunately this information, while helpful in rating materials, is difficult to translate into results expected from field applications. The effect on the colony depends on formulations, repellency, time and method of application, systemic effects, and a number of other factors which cannot be duplicated in the laboratory.

One of the earliest field tests with organic insecticides in relation to honey bees was conducted by McGregor & Vorhies (40), who determined that DDT [1,1,1-trichloro-2,2-bis (*p*-chlorophenyl) ethane] could be used on cotton fields without serious economic loss to honey producers. These results have been confirmed in practice, and DDT is commonly used as a standard for comparing other insecticides. Materials more toxic than DDT are considered too lethal for use around bees.

Field testing of insecticides on honey bees is more difficult than on most other insects. The objective is to determine damage to the colony, its field force, and its pollinating or honey-production potential. Bees make brief visits to a field rather than becoming residents; this complicates evaluating the effect on the colony which may be some distance away from the field. Test fields must be carefully chosen to be reasonably certain that bees from the colonies under observation are actually working in the treated area. At best, part of the field force may be working elsewhere, and results obtained may be influenced by various factors beyond the borders of the test field. Furthermore, no satisfactory method has been developed for measuring accurately the sublethal effect of an insecticide application on a colony.

Testing techniques have been developed by the United States Department of Agriculture Bee Culture Laboratory at Logan, Utah [Lieberman *et al.* (35); Todd *et al.* (61)] and the University of California at Riverside [Anderson & Atkins (1)] for estimating the effects of insecticidal treatments of crops on honey bees. Caged bees are placed in the path of the applicator to test the effect of direct applications. The fuming effect is tested by placing additional cages in the field at hourly intervals until no more mortality occurs. The effects on the visitors to the field are estimated by counting visitors

before and after the application in treated and untreated areas until the visitation level returns to normal. When most of the visitors are killed, scout bees must recruit a new field force for the area. Repopulation of the field with bees will not occur until scout bees can survive visits to the flowers.

Estimates of the populations and amount of brood in colonies located in sprayed and unsprayed areas are determined before and after application to evaluate the effect of treatment on either the brood or the colony populations. In front of these hives, a measured ground area is cleared from which dead bees are picked up daily and counted. Precounts are made to establish the normal mortality and the counts are continued until the mortality returns to the preapplication level. These data provide a basis for an estimate of the death rate at the colonies as well as indicating the degree of residual effect of the material.

The combined data obtained from the cage-bee tests, observations on field population, hive population, conditions of the brood, and mortality counts in front of the hives collectively provide a fair estimate of the effect on the colony of field applications of insecticides.

A current appraisal of the relative toxicity to bees of most of the insecticides, defoliants, fungicides, and weed sprays commonly used, based on data given by Anderson & Atkins (1), Johansen (31), and Palmer-Jones (48), is shown in Table I.

The modern insecticides, unlike arsenicals, have their greatest effect on the field bees. Those bees contacted within the field or in the air during the application are most likely to be killed. The fuming action of some materials may cause additional mortality. With few exceptions (dieldrin and Sevin) the residual effect on bees is of short duration, usually not more than 24 hours. Damage to brood is usually light, unless the colony is so seriously disorganized that the brood is neglected and it dies.

Lieberman *et al.* (35) set a 10 per cent mortality of the field force as the maximum tolerance for sanction of a bloom-stage application of insecticide. He also pointed out that the seriousness to the beekeeper of the mortality lies not alone in the percentage killed but also its time in relation to the honey flow. The beekeeper's share, the surplus honey produced, is only a small proportion of the total amount of food carried into the hive, probably not more than 20 to 50 per cent [Ribbands (55)]. The loss of field bees during the honey flow cuts into that portion. A small loss of bees is greatly magnified in terms of lost honey production.

Knowledge of the relation of insecticides to bee poisoning has suggested the idea that a temporary closing of the hive may greatly reduce the hazard; This has yet to be proven. According to Chauvin (12), bees withstood three days of confinement very well, but repeated confinements were unsatisfactory. Placing a screen between the bottom board and brood chamber gave the best results. Bogoyavlenskii & Merits (4) reported on some hive-closing experiments with confinement up to 10 days. They concluded that the best

TABLE I
TOXICITY OF PESTICIDES TO HONEY BEES

Group 1			
<i>Highly toxic to bees; direct application kills all bees, and the treated plants remain toxic 24 to 48 hours.</i>			
aldrin†‡	DDVP*	DNOSBP (DN-211)§	lindane†‡
BHC†‡	Diazinon*§	EPN†‡	Metacide‡
calcium arsenate‡	Dibrom*‡	Guthion‡	methyl demeton§
chlordane†‡	dicaphon*	heptachlor†	parathion*†
Chlorthion*	dieldrin*†‡	lead arsenate‡	Sevin*‡
Group 2			
<i>Highly toxic to bees; direct application kills all bees, but plants are safe after 4 to 6 hours.</i>			
Di-Syston*	Phosdrin*‡	TEPP†‡	
malathion*†‡	sabadilla*	Thimet‡	
Group 3			
<i>Moderately toxic to bees; can be used around bees if timing and dosage are correct, but should not be applied directly on the bees in the field or at the colonies.</i>			
Chlorobenzilate*	DDT*†‡§	Perthane‡	Thiodan*§
Cryolite‡	endrin*†‡	Strobane§	toxaphene*†‡§
DDD(TDE)‡	ethion (Nialate)*†	Tedion*	Trithion*
	isodrin*		
Group 4			
<i>Relatively nontoxic to bees; can be used around bees safely.</i>			
allethrin*	demeton	Kelthane*	rotenone‡
Aramite‡	(Systox)*†‡	Maneb*§	schradan (OMPA)‡
Bordeaux mixture*§	dilan‡	MCP*§	silica gel*
Captan*§	DMC (Dimite)*†‡	methoxychlor†‡	sulfur‡
chlorbenside*	DNOCHP (DN-111)*	Monuron (CMU)*§	Sulphenone‡
copper sulfate		nabam*§	Systox (demeton)*†‡
(monohydrated)*	Dylox (Dipterex)*†‡	Neotran‡	2,4,5-T*
Cunilate*	Endothal*§	nicotine‡	thiram*§
cuprous oxide*§	Genite*	ovex (Ovotran)‡	zineb*§
2,4-D§	IPC*	Phostex*	ziram*§
Delnav*†‡	Karathane*	Pyrethrum‡	

Field tests reported by:

* Anderson & Atkins (1), † Lieberman *et al.* (35), ‡ Johansen (31),
§ Palmer-Jones (48).

The following abbreviations are used in Table I: Aramite for 2-(*p*-tert-butylphen-
oxy)-1-methylethyl 2-chloroethyl sulfite; BHC for 1,2,3,4,5,6-hexachlorocyclohexane;

method was to close the entrance completely, and to use a top ventilating screen with enough room for the bees to cluster and to provide the colony with plenty of readily accessible water. The Bee Culture Laboratory at Tucson has made a few confinement experiments but only for periods of eight hours. The colonies were not materially affected by the treatment. The effect of closing colonies located in the sun or under conditions of high temperature is unknown. The knowledge we have gained from the study of insecticides indicates little need for closing the hives more than 24 hours. For many materials, confinement during the time of application only may be sufficient.

Effective use of honey bees as a tool in agricultural crop production is apparently in its infancy. At the present time, growers of fruit and small-seeded legumes are the principal users, although there is a long list of other crops known to be benefited by insect pollination. Research is continually

Captan for *N*-trichloromethyl-mercapto-4-cyclohexene-1,2-dicarboximide; Chlorobenzilate for ethyl 4,4'-dichlorobenzilate; Chlorthion for *O*-(3-chloro-4-nitrophenyl) *O,O*-dimethyl phosphorothioate; Cunilate for copper 8-hydroxyquinolate; 2,4-D for 2,4-Dichlorophenoxyacetic acid; DDD (TDE) for 1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethane; DDT for 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane; DDVP for 2,2-dichlorovinyl dimethyl phosphate; Delnav for 2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate; Diazinon for *O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate; Dibrom for *O,O* dimethyl *O*-(1,2, dibromo, 2,2 dichloroethyl) phosphate; Dicapthon for *O,O* dimethyl-*O*-2-chloro-4-nitrophenyl thiophosphate; Di-Syston for *O,O*-diethyl *S*-2-(ethylthio) ethyl phosphorodithioate; DMC for *p,p'*-dichlorodiphenyl methyl carbinol; DNOCHP (DN-111) for 4,6-dinitro-*o*-cyclohexylphenol; DNOSBP (DN-211) for 4,6-dinitro-*o*-*sec*-butylphenol; Dylox (Dipterex) for *O,O*-dimethyl-1-hydroxy-2,2,2-trichloroethyl phosphonate; EPN for *O*-ethyl *O*-*p*-nitrophenyl phenylphosphonothioate; Ethion (Nialate) for bis-[*s*-(diethoxyphosphinothioyl) mercapto] methane; Genite for 2,4-dichlorophenyl benzenesulfonate; Guthion for *O,O*-dimethyl *S*-(4-oxo-3H-1,2,3-benzotriazine-3-methyl) phosphorodithioate; IPC for *N*-phenyl isopropyl carbamate; Karathane for 2-(1-methylheptyl)-4,6-dinitrophenyl crotonate; Kelthane for 1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethanol; Maneb for manganese ethylene bis-dithiocarbamate; MCP for 4-chloro-2-methylphenoxyacetic acid; Metacide for a formulation of methyl parathion and parthion; Mitox for *p*-chlorobenzyl *p*-chlorophenyl sulphide; Monuron (CMU) for 3-(*p*-chlorophenyl)-1,1-dimethylurea; Neotran for bis(*p*-chlorophenoxy)methane; OMPA for octamethyl pyrophosphoramide; Perthane for 1,1-dichloro-2,2-bis(*p*-ethylphenyl)ethane; Phosdrin for 1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate, 60 per cent technical; Phostex for a mixture of bis(dialkylxyphosphinothioyl) disulfides; Sevin for *n*-methyl-1-naphthyl carbamate; Sulphenone for *p*-chlorophenyl phenyl sulfone; 2,4,5-T for (2,4,5-trichlorophenoxy)acetic acid; Tedion for 2,4,4',5-tetrachlorodiphenyl sulfone, 80 per cent technical; TEPP for ethyl pyrophosphate, approximately 40 per cent technical; Thimet for *O,O*-diethyl *S*-(ethylthio)methyl phosphorodithioate; Thiodan for 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide; Trithion for *S*-(*p*-(chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate.

expanding this list. The trend toward the utilization of hybrid vigor in more crops suggests there will be a still greater need for honey bees for pollination. The gradual elimination of native pollinating insects by modern agriculture practices has increased the grower's dependency on the honey bee.

Considerable progress has been made in the use of honey bees for pollination since Waite (66) recommended that sufficient bees be within two or three miles of orchards. If such progress continues, and it is dependent on the solution of problems discussed herein and many others, the bee industry, which is presently supported by honey production, may soon find pollination service its primary concern.

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THE BIOLOGICAL BACKGROUND OF LOCUST CONTROL¹

By D. L. GUNN

International Red Locust Control Service, Abercorn, Northern Rhodesia²

There is no single species properly named "locust," for intensely migratory and swarming species, like the desert locust of the Bible, shade off into sedentary and nonswarming grasshoppers, some of which cause local damage. Few generalizations are applicable to such varied species. Not one has yet been deliberately brought under complete control by ecological methods; but the Rocky Mountain locust [*Melanoplus spretus* (Walsh)] has been accidentally discouraged by man during the colonization of the North American prairies and may be extinct (10); on the other hand *Locusta* and the Moroccan locust [*Docostaurus maroccanus* (Thunberg)] have been encouraged by man (131). Although ecological methods of control are being sought (4, 26, 46, 76), at present all effective and economical control depends on chemical insecticides; it is unlikely that ecological control will soon, everywhere, oust chemical control, so the biological background of the latter is worthy of consideration and improvement in its own right. Both the strategy and the tactics of chemical attack depend on the biology of the species concerned; they also depend on finance and politics.

PHASES

Fifty years ago, different specific names could be given to the parents and the offspring of an individual locust. This led to misunderstandings about the genesis of plagues, which were resolved by Uvarov's discovery of locust phases. He states that each locust species is plastic, both morphologically and biologically; of the extreme forms, or phases, previously regarded as distinct species, the one (*gregaria*) behaves as a swarming locust, the other (*solitaria*) like a solitary grasshopper (126, 127). It was at first thought that change to phase *gregaria* would greatly increase the multiplication rate, but it does not generally do so—not, at any rate, for the larger species, on which it sometimes has the opposite effect (16, 80, 81). Weather factors, on the other hand, can alter multiplication rates over a very wide range, and it has never been shown that variations in multiplication associated with phases are in the same class of importance.

The main facts are well known (57, 61). Behaviour changes are clearly vital in control, for it is the concentration of locusts into migratory nymphal bands and adult swarms that make them dangerous; it is also this behaviour that makes control with insecticides economical. But dense gregarious and migratory swarms of *Schistocerca gregaria* (Forskål) and *Chortoi*.

¹ The survey of literature for this review was concluded in October 1958.

² Present address: Tea Research Institute, Talawakele, Ceylon.

celes terminifera (Walker) have sometimes been found to be morphometrically *solitaria* (32, 55, 62), and although, in the laboratory, chromatically *solitaria* nymphs aggregated and marched less than *gregaria*, the differences required exact statistical treatment to reveal them, and they diminished to very little after a few days crowding of *solitaria* (29, 30, 31). That is to say, the insects remained for a time morphometrically and chromatically *solitaria* but behaved in a *gregaria* manner if crowded. Consequently phase status, as indicated by form and colour, does not reliably indicate either present or future behaviour. In fact, behaviour sometimes changes too rapidly for form to change with it, while form does not change at all in adults, though behaviour may do so.

Partly because of the confusion caused by having independently varying criteria of form, colour pattern, and behaviour for phase status, Key, in a masterly review of Uvarov's phase theory and its accretions up to 1947, decided in favour of morphometric criteria alone (61). Because the word "phase" is in common use, locust phases are distinguished as "kentro-morphic" (64). Thus the terms *gregaria* and *solitaria*, apparently describing behaviour, actually refer to morphological types.

Although present and future behaviour cannot be inferred from morphology, something about past behaviour might be, and this might lead to a better understanding of how certain situations tend to arise. What, then, are the conditions determining morphometric phase status? Faure showed that the degree of crowding during nymphal life is a major factor (33), but this alone has not produced, in the laboratory, populations of both extreme forms. There are also three different kinds of inherited factors in *Locusta migratoria migratorioides* (Reiche & Fairmaire): (a) four generations of selection for high and for low wing/femur ratio, using locusts kept at constant medium density, produced locusts just as extreme on this criterion as did crowding and isolation, and the effects of crowding and of selection were additive (47); (b) selection for decreased response to crowding and isolation has also succeeded (147); and (c) isolation or crowding of the female parent (of whatever phase) affects the egg and the phase status of both the hatchlings and the resulting adults, again additively (1, 51). Environmental factors, other than crowding, which tend to produce variation in the direction of *gregaria* forms in various species are low temperature, low air humidity, and kind of food (10, 61). That is to say, it will probably always be extremely difficult to judge, from the morphometric phase status of a population, what the exact history of that population is likely to have been, though with some species broad inferences may sometimes be reliably drawn. A very refined analysis of characters in *S. gregaria* may help in deciding certain critical theoretical questions (122). It is less likely to be useful in routine control.

The principal success of the phase theory has in fact remained the identification and localization of *solitaria* forms from which a plague of *gregaria* may arise; the most recent example is the South American *Schisto-*

cerca paranensis (Burmeister), with its *solitaria* form, formerly called *S. cancellata* (Serville), though there is still disagreement about this (13, 20, 82, 90).

Locust species are extremely variable, but there seems to be less variation within a population from a limited area than between populations in different areas. Using every kind of measurement, including those of phase status, it is sometimes possible to establish that two populations have different origins (23, 102).

The American genus *Schistocerca* is represented in the Old World by the desert locust, *S. gregaria*, whose plague area extends over southwestern Asia into Africa somewhat south of the equator. In South West Africa, completely isolated from this plague area, there is a population provisionally called *S. gregaria flaviventris* (Burmeister) which is interfertile with typical *S. gregaria* (52), but is comparatively static, requiring little control (28, 34). It may be that conditions are too unfavourable for large numbers to be reached, though densities sufficient to break branches off trees have been recorded (38); but it is noteworthy that the South West Africa variety is considerably less affected in its morphometry by crowding in the laboratory (52). This is not in itself an indication of probable behaviour; but both extreme morphometry and migratory behaviour may have been selected out, by migration to places from which no return could occur, so that the most migratory individuals would always be culled (65, 148). The idea can be carried further: continual attack with insecticides on bands and swarms of a species in an outbreak area may eventually reduce the strength of gregarious behaviour by selection and thus lead to a population that may become large but remains dispersed and nonmigratory—a perennial but local pest, like some species of *Melanoplus*. There would be no objection to such a development in the outbreak areas of *Locusta* and *Nomadacris* in continental Africa, which are in nonproductive lands.

Key's restriction of the word "phases" to morphological types is not agreed to by Uvarov, nor by Kennedy (57). Kennedy maintains that changes in form and colour pattern are evidence of profound physiological changes, which must be taken into account if locust plagues are to be understood. It is generally agreed that behaviour changes are crucial in the rise of a plague, but it is not generally agreed that it is practically convenient to use the word "phase" to cover variations in behaviour as well as in form. Kennedy does agree, however, that research has been unduly concentrated on phases, to the neglect of multiplication and survival; whatever the use of the word "phase," it is in quantitative population dynamics that advances are most urgently needed.

OUTBREAK AREAS

The first strategic conception for economical control of a locust plague was based on the discovery that the plague of the Rocky Mountain locust (*M. spretus*) during the 1870's originated in a permanent breeding area of

300,000 sq. miles and spread over an invasion area of 1,000,000 sq. miles more (107). Successful control in the permanent breeding area would obviate all control elsewhere. It became evident much later, however, especially for *Locusta migratoria* (Linnaeus), that breeding in the *solitaria* phase could go on indefinitely in many places where it never reached outbreak dimensions, and by 1934 attention had been focused on those permanent breeding areas in which outbreaks occurred, which were named "*foyers primitifs*" (19), "outbreak areas," or "*aires grégarigènes*" (53). Definite outbreak areas had long been known for *L. migratoria migratoria* (Linnaeus) in Russia and were found about 1930 for *L. migratoria migratorioides* and the red locust, *Nomadacris septemfasciata* (Serville), in continental Africa (130). For the last, the outbreak areas total less than 2000 sq. miles, and the ratio of extent of plague area to outbreak area is about 1500 to 1 (45). There are now permanent outbreak-area control organizations, paid for internationally, which have been quite effective in preventing plagues (45, 151). In Russia, there is a permanent "expedition" to prevent plagues of *L. migratoria migratoria* (54).

In strictest usage, the expression "outbreak areas" for a species of locust refers to those special places in which plagues of the species always originate from dispersed resident populations. After over a decade of search, no outbreak areas, in this sense, have been found for *S. gregaria*, and the idea that they exist at all is untenable (36). Indeed, in every year since 1887, at least one swarm has been reported from somewhere in the vast plague area (97), so at first there seems no need to postulate the existence of outbreak areas, but merely of a perpetual state of swarming. There is one indication that new life may be given to this chronic plague by locusts that do not arise from previous swarms: after a recession, the early swarms may be of morphometrically *solitaria* locusts, suggesting that they arose by concentration of a scattered population (32). On the other hand, locusts that are reported as a swarm in a period of recession may be sufficiently scattered to produce *solitaria* progeny and, further, under hot conditions *solitaria*-like adults may derive from crowded hopper bands (122).

According to the latest conception arrived at in the Anti-Locust Research Centre, London, the upsurge of a desert locust plague, after a period of recession, may arise either from carry-over of swarms or from "the concentration of scattered locusts, as a result of meteorological factors, into areas ecologically suitable for successful breeding and gregarisation. The location of such areas might vary according to the weather systems operating at the time." Such areas have been named "concentration areas" and "gregarisation areas" (36). Their importance is considered not to depend primarily on the pullulation of a resident population, and the term "gregarisation areas" is likely to cause confusion with the French name for outbreak areas, namely "*aires grégarigènes*." Quantitative work may indicate the relative importance of concentration and of successful localized reproduction, though the areas involved are vast.

Until recently, the South American plague species of the same genus, *S. paranensis*, had been swarming, with fluctuations, for about 60 yr. The northern Chaco, on the borders of Argentina, Bolivia, and Paraguay, has been suspected as an outbreak region (13, 20, 70, 90). The opinion that *S. cancellata* is phase *solitaria* of *S. paranensis* may intensify search for outbreak areas, but the situation may turn out to be similar to that for *S. gregaria*.

Between *Nomadacris* (and *Locusta*) and *Schistocerca*, there are other species more like *M. spretus*. Thus in the brown locust of South Africa, *Locustana pardalina* (Walker), outbreak areas occur anywhere within 130,000 sq. miles, while the plague area is about 330,000 sq. miles (28). In the Australian plague locust [*Chortoicetes terminifera* (Walker)], the relationship is much the same (59).

The Moroccan locust is still more local in its invasions. All around the Mediterranean and eastward to Central Asia, it lives, with some exceptions, on denuded stony hillsides (8, 76, 128, 138). After successful breeding, it may migrate to lower, cultivated, lands at a season when crops are growing. Normally migration does not go further than 60 miles (100 km.). The problem is thus essentially a local one, though it may cross political frontiers. Nevertheless, outbreak-area control may be applicable, for Pasquier holds that all damage can be prevented by units of a man, a donkey, and a bag of bait, applied at the right times and places (88).

As for the basic nature of outbreak areas, the obvious idea is that they are places where reproduction and survival are high, but it has been stated that "the old conception that the outbreaks commence in the areas and seasons with the most favourable conditions for the species" can be safely disposed of (129); emphasis was placed on the intervention of dry conditions in concentrating the locusts, leading to phase *gregaria*. Key has criticized the idea that dry conditions are necessarily unfavourable (60, 61); Acrididae, including locusts, are usually successful in arid and semiarid zones (131); in the red locust, concentration occurs after successful breeding and begins before there is any drying up or perceptible deterioration of the habitat (44, 113); and the initial migratory swarms of the red locust, as far as they have been investigated, are not of phase *gregaria* but are *transiens* and require very detailed investigation to distinguish them from the normal resident population (102).

It has been suggested that the peculiarity of outbreak areas of the red locust is that gregarious behaviour in them is inhibited by the dense vegetation, so that high numbers accumulate before emigration is released (102). Although this effect of dense vegetation has been observed with desert locusts (32, 55) and may occur in the red locust, such data as are available for red locusts indicate that it is only in the recognized outbreak areas that really large numbers are ever produced during nonplague periods; and from them alone does swarm emigration occur. Given sufficient numbers, concentration and gregarious behaviour will occur; if the population is not so big, coherent and persistent but nonmigratory swarmlets tend to form (102, 113). No

doubt the patchy vegetation of most outbreak areas assists concentration, but variable patchiness is a common feature of arid and semiarid zones and is favourable to survival by offering a variety of ecoclimates (61). The view that outbreak areas are essentially places that are outstandingly favourable to reproduction and survival has been expressed for Moroccan locusts, Australian plague locusts, and African migratory locusts, as well as red locusts (8, 23, 59).

For *L. migratoria migratorioides* in Africa, the outbreak region contains a series of areas which are used cyclically every year by succeeding generations of locusts, which migrate among them. Here, there is an outbreak-area system more complex than just a favourable place to breed and survive (21, 22, 106). Again, even if some part of the South West Africa area of *S. gregaria flaviventris* were very favourable in itself, if there were no suitable plague areas from which it could be re-infested, it could hardly constitute an effective outbreak area (see p. 281). A plague area, too, may require more than merely favourable conditions for survival and reproduction; with several African species that exhibit prolonged plagues, the meteorological conditions are suitable in that they enforce migrations of a cyclical or to-and-fro nature and in general keep the swarms within suitable conditions over periods of years or decades.

MIGRATION

Suppose that there were about 10^{11} adult red locusts weighing 300,000 tons at the 1934 to 1935 peak of the last plague (see p. 287 below). Such a number would soon consume the total tonnage of edible grass growing in the 2000 sq. miles of outbreak areas. Swarm emigration from the outbreak areas may, therefore, from time to time enable a greater mass of the species to be produced than could survive within the outbreak areas. That is not to say that lack of food is a proximal stimulus to emigration, for in this species departures begin when there is still an abundance of edible grass. Emigration diverts a Malthusian crash (12) and is essential to the fullest success of any highly gregarious animal (31).

It is this emigration in swarms from economically unimportant areas to crop areas, producing sudden and complete destruction, that has made locusts important from the earliest times. The gregarious behaviour not only makes locusts economically important; it also enables them easily to find mates far from the normal habitat, and it facilitates economical attack with insecticides. For example, one swarm was destroyed at the rate of 3,000,000 locusts, weighing about 6 tons, per Imperial gallon of 85 per cent Diazinon, while up to 1/2 ton per gallon was commonly achieved with various insecticides (95, 97, 98).

Migration by individual locusts, as distinct from swarms, occurs in *L. migratoria* in Europe (140). It is an essential part of the annual cycle of the African subspecies (21, 22, 106), and the discovery of the cycle has led to a complete re-organization of the system of population assessment and con-

trol. Individual migration is probably a vital component in the survival of the desert locust (104, 105). So far, the evidence is against much emigration of separate red locusts from an outbreak area (113).

For swarms, it would be most valuable to be able to forecast where they will go; this is particularly important for the desert locust, which seems to have no outbreak areas and which largely breeds in places where it does no harm. The problem has been tackled by the Anti-Locust Research Centre, London, in two ways: first, for thirty years information has been collected, mapped, and analyzed, so that it is now possible to say how a plague may spread, on the basis of what has previously happened; and second, the factors of weather and locust behaviour that result in the swarm movements that are observed have been analyzed. Movement of desert locust swarms occurs in a down-wind direction (92, 100, 105, 141); in the north African and southwest Asian region where the species flourishes, this results in seasonal to-and-fro or cyclical migrations that typically bring the swarms into the seasonal rains of the inter-tropical convergence-zone in summer and of westerly depressions along the Mediterranean and Persian Gulf in winter and spring, where seasonal breeding occurs (25, 27, 36, 37, 131). Winds can produce these results only if the locusts take flight and behave in certain ways, which have been disputed and elucidated by several authors (48, 56, 93, 96, 101, 142, 143, 144). It is possible that similar results will be found for the South American swarming species of *Schistocerca* (20).

The cartographical summaries of past migrations have been successfully used to give warning of impending invasions; the analytical work on behaviour has mainly served to explain what has already happened. More successful forecasting of detailed weather is needed to make the best use of the behaviour work, but some use is possible. Synoptic weather information is more rapidly available than detailed news about desert locusts in this vast and sparsely inhabited plague area, and it can sometimes indicate what is likely to be happening to swarms and their breeding, and where they should be sought (94). It may be possible to improve the utilization of weather data for this purpose (36, 96).

POPULATION DYNAMICS

The expression "population dynamics" is tending to become a mere catch phrase and therefore to lose precision; it is here used to mean the study of the sizes of the "forces" acting on a population and their quantitative effects on that population. Kennedy quite fairly summarizes the shift of interest from phases to "mere numbers" and he questions whether "population changes and phase changes can be separated in nature or whether they are so interdependent that neither can be studied without taking full account of the other" (57). Locusts (but not other Acrididae) change their behaviour upon crowding (and later change in other respects) and it is this behaviour change which makes them so dangerous. But within one species of locust, of the two variables, numbers and phase status, numbers are almost independent of phase,

and phase is almost completely dependent on numbers. Uvarov himself states, without particularizing, that "the importance of some aspects of phase variation may have been over-estimated" (132). We should therefore not be preoccupied with phases but should study the changes in the numbers and distributions in complete populations of locusts in the field and the causes of these changes. Behaviour will affect distributions once the numbers are there. Phase, whether kentromorphic or deep-seated, will come into true perspective when more is known of the quantitative aspects of outbreaks and plagues.

The quantitative approach is in its infancy, and little has yet been done except exploratory study of methods. It has been customary to assess locust populations by counts of densities, particularly where densities are high, but often with little indication of the extents of the areas concerned; more thinly dispersed populations may be larger, because much more extensive, and may cause surprise by concentrating (105).

Red locust populations in the outbreak areas, except when swarms emigrate, are effectively isolated; total numbers and distributions of adults are estimated for complete outbreak areas by sampling in a vehicle along carefully placed line-traverses, counting locusts that fly up in a strip as wide as the vehicle (113). Consistency is generally achieved within 20 per cent, but the clumpy nature of the distribution makes statistical estimates of random errors uncertain at present; attempts are being made to reduce systematic errors. The results show quantitatively that concentrations of adults disperse just before oviposition, and there is no early formation of dense bands from gregarious laying, such as occurs with the desert locust (91), nor from asynchronous selection of "nests" as in the brown locust (119). Red locust nymphs do behave gregariously, if dense enough, but evidence has been sought and not found that this behaviour leads to any great degree of fusion of bands after the third instar (112). When the adults emerge, however, successive estimations of density distributions show that the frequency of zeros rises and the number of locusts at high densities increases progressively. If a concentration thus formed is both large and dense enough, swarm emigration begins; present data suggest 5 to 10 million locusts as the minimum content of an emigrant swarm. Such emigration can occur within a month of the first adult emergence, before there is any perceptible deterioration of the habitat resulting from grass fires or the advancing dry season, or at any time up to shortly before egg-laying dispersal, and it must be regarded as a behavioural response to high densities and numbers. Swarms so formed arise from individuals with a great variety of antecedents, and it is therefore not surprising that morphometrically they are not in phase *gregaria*, but are *transiens* with a broad scatter of measurements and do not appear to be derived from any simple mixture of populations of markedly differing morphometrics (102).

Within an outbreak area of 100 to 550 sq. miles, mortality of adults through the long dry season may be almost nil, as far as the accuracy of the

methods shows, or as high as 90 per cent. Breeding, which is closely followed by the death of the parents, may hardly increase the population or may give a natural increase of 45 times, reckoned as the ratio of offspring killed and assessed as corpses plus survivors to parents (68, 69, 113). When the increase is large, control measures have to be taken, so it is not then possible to find out what the increase of breeding adults may reach in successive years, with only natural mortality intervening.

Because of recent success in preventing plagues of the red locust, it is not likely to be possible to assess the numbers in a plague population, so we have to use such data as exist for the plague of 1929 to 1944 (43, 77) and recent data for the outbreak areas (68, 69, 113). Just before the breeding season in both 1957 and 1958, the total number of adults in the 2000 sq. miles of their outbreak areas was approximately 2×10^7 ; in 1953 there was the same number in the North Rukwa outbreak area alone, while in 1955, just after the completion of hatching, the number there was at least 1.9×10^9 (69).

For the last plague (1929 to 1944), there are no records of numbers of locusts, only numbers of swarm reports. Assuming that these correspond to relative numbers of swarms of standard content, the increases in the two breeding seasons before the emigrations from the outbreak areas of 1929 and 1930 totalled 140 times; thereafter, to the peak of 6300 reports in 1934, the maximum in 1 yr. was four times and the total in 4 yr. about 45 times, thus averaging only 2.9 times per annum. That is to say, the big increases took place within the outbreak region, and comparatively small but repeated increases in the plague area were sufficient to lead to an enormous and sustained plague. This is consistent with the idea that the outbreak areas are particularly favourable to the locusts and that a plague dies out because the invaded areas are not persistently so [cf. pp. 284, 285; Bodenheimer (8)]. Indeed an outbreak may fail at the start if the invaded area happens to be unfavourable at the time (45).

There are no data for other species on fully self-contained outbreak or plague areas. A single concentrated oviposition from one swarm of desert locusts gave 20 times as many hatchlings as parents (123); the swarm probably laid elsewhere as well and though some swarms would fail to reproduce at all, multiplication of *Schistocerca* during a plague may match that of red locusts in its outbreak areas. Rainey estimated that 500 sq. miles of swarms of desert locusts invaded Kenya in January 1954 and, by assessing corpses after aircraft spraying, he found that there were probably between 10^{10} and 5×10^{10} locusts, which may be approaching the total number for the peak of a red locust plague (perhaps 10^{11}). He also stated that a single swarm could contain 10^{10} individuals (93, 96, 98). MacCuaig estimated the total number of desert locusts in Somaliland in August 1957 at 1.6×10^{10} ; at one third of a million to the ton, this number would weigh 50,000 tons (73). Growing or migrating locusts eat about their own weight in green food per day (24, 81, 146); both the cause and the danger of a plague arise from sheer magnitude.

The African migratory locust migrates individually in its outbreak areas

so that there is an annual swing of the population between complementary flood-plain and semidesert areas (22, 106); locusts and plague grasshoppers that have a more limited range of movement seem to be more promising material for quantitative study, but even with the Moroccan locust, Dempster had trouble with emigration of bands (26). Enumeration methods must be varied for the different species and their accuracy will also vary.

A full-length review of qualitative aspects of population changes would be valuable. Analytical work in laboratories indicates rates of increase and suggests factors that may be important in the field (51, 80, 81). There seems to be a direct relation between size of female and number of ovarioles (139) or number of eggs laid (8), and, in so far as size is a phase character, phase may affect reproductive rate to some extent (1, 16). Possibly the most important single factor is the length of the breeding season, as determined by weather at its start and end (2, 23, 26, 108).

Once the eggs are laid, one expects the mortality of 10 to 40 per cent that is common in insects, as a result of failure of fertilization, lethal genes, and so forth. Losses are caused by drought at the time when the embryos must take up water and by infections in excessively damp conditions. In *Nomadacris* and the tropical subspecies of *Locusta*, the eggs must hatch in a few weeks or they die. Some species are assisted by diapause eggs or by prolonged dormancy, broken by rain; in the brown locust hatching may extend over many months, and this complicates analysis of population changes (75). Some losses are common from predation and parasitism, but these rarely seem to be crippling (26, 87).

Once the locusts have hatched, their numbers are more exposed to reduction by weather, and by predation and parasitism. Cool, damp weather is known to be unfavourable to *Melanoplus* species and is probably so for most short-horned grasshoppers; red locusts, on the other hand, have been seen to hatch and die almost at once in very hot dry conditions. If locusts are concentrated and in large numbers, they are attacked by a great variety of birds, mammals and reptiles, but in such circumstances the sheer tonnage of locusts is often so great that the proportion destroyed seems to be trivial. Little is known of the extent of natural mortality and still less of the causes. The brown locust, with an adult life of about three months, may lose 36 to 52 per cent in a month (119), while in red locusts, over the dry season, the losses may be great or small (113).

Predators and parasites seem rather unimportant for locusts, but weather seems to be important at every stage. In outbreak areas, the only important density-dependent processes (121) suggested are swarm formation and its results, namely chemical control and swarm emigration (57, 79). If other density-dependent processes were important in swarming locusts, then chemical control might merely take the place of natural control of numbers and might indeed lead to a higher level of population at considerable cost; but certainly natural control is not always effective, and important natural density-dependent processes are not conspicuous either in outbreak areas or

in plague areas. Since there is certainly some natural mortality at all stages in the life history, chemical control may destroy locusts that would die harmlessly, but it is unlikely to make the situation worse, as it might do if it usurped the place of some kinds of natural density-dependent process. Carefully timed operations, guided by quantitative knowledge and understanding of natural mortality, may reduce useless killing (68). Further work is needed, possibly to contribute to population theory, but also to facilitate forecasting and economical control.

FORECASTING

Locust infestations are characterized by violent fluctuations in size: in many years, no control is required; in others, for successful control, such a large concentration of resources may be required—human, financial, and material—as to upset the economy of a whole territory. To save the waste of unnecessary preparations or the ineffectiveness of last-minute action, forecasting would be very valuable. There is no evidence of any regular periodicity that can be used (18).

It may be possible to foresee the upper limit of either (a) the future numbers (and resulting behaviour) of existing individuals or (b) the numbers of the first filial generation, as yet unconceived. In either case, ascertaining the size of the existing (or parental) population is essential, for this sets the upper limit to the future population. Future weather cannot be forecast sufficiently in advance and therefore leaves a substantial uncertainty. Forecasting has therefore generally to take the most pessimistic line; if it is then favourable, savings may be made with confidence.

The most widely used assessment in the first class is based on planned sampling of soil for dormant eggs in the autumn, indicating the maximum population possible after hatching in the following spring. Such sampling is regularly done for grasshoppers (mostly *Melanoplus* species) over a vast area (115) of the United States and Canada and in Russia (118), and also for the Moroccan locust in Spain (14). This method is applicable only where there is prolonged egg dormancy with a definite hatching season and is unsuitable for species in which hatching begins before the last eggs are laid and for the brown locust of South Africa, with delay in hatching that may extend to two years. It requires a large and reliable staff and must therefore be justified by immediate savings in valuable crops.

Another kind of one-generation enumeration is used with the red locust, which has a prolonged adult diapause. Enumeration begins as soon as adult emergence is complete (113); at this stage, the locusts are often too dispersed for attack or for qualitative assessment by experience, but during the following months they congregate and tend to form migrant swarms. The determination of numbers early in the season enables the degree of danger to be forecast objectively and plans can be made accordingly.

An entirely different kind of forecast is required for a swarming and migrating species during a plague. The seasonal pattern of swarm migration of

the desert locust forms an empirical basis for an international warning system maintained by the Anti-Locust Research Centre since 1943 (25, 27, 37, 77, 141; see p. 285 above).

The most valuable kind of forecasting would have to be more far-sighted. Among the causes of large population fluctuations, weather is undoubtedly a major factor. The Rocky Mountain locust plagues were associated with drought, especially over the previous two or three years (107). Uvarov quoted Russian authors from 1870 onward who associated outbreaks of Acrididae with hot and dry weather (127). *Melanoplus mexicanus* (Saussure) prospered in dry years but other species did better in wet years (135). The drought of 1934 to 1938 preceded the upsurge of *M. mexicanus* which led to mass flights in 1938 and 1940 and consequent crop losses valued at sixty million dollars (86, 115). Wakeland showed that outbreaks of *Dissosteira longipennis* (Thomas) of the High Plains from Texas to Nebraska followed periods of drought (137). Even species that flourish in floodlands show a similar association; for example, plagues of *Locusta migratoria manilensis* (Meyen) in both the Phillipine Islands and China tend to follow drought (84, 125). The important Russian subspecies, *L. migratoria migratoria*, tends to increase greatly in prolonged droughts or after poor spring floods; forecasting is based on the height of these floods (117, 150). For the red locust, in poorly recorded history an association was found between dry periods of years and locust upsurges in flood-plain outbreak areas (43, 44).

An extensive study of such relations was made by Smith for 100-year records of often unspecified species, probably mostly *Melanoplus*, in Kansas, U.S.A. (120). He concluded that "grasshoppers have increased in dry years, as shown by the population in the year following; outbreaks have followed two years in succession of subnormal rainfall," but these conclusions were marred by the impossibility of distinguishing accounts of different species, which may respond differently (3, 89).

MacCarthy investigated more accurate records of a single species (*M. mexicanus*) over 10 years in Saskatchewan, Canada (71). He found correlations between the weather up to 14 months earlier and a population size index; most of the correlations tended to be uncertain and not applicable to all the areas studied, so that no very simple method of forecasting could be put forward. Any relationship was likely to have been upset by the introduction of the chlorinated hydrocarbon insecticides during the decade.

For the brown locust in South Africa, it has been held that outbreaks are preceded by one or more seasons of poor rainfall (35, 119). Lea made a statistical study of outbreaks in connection with variations in rainfall from 1941 to 1955 (66), using the cost of control in a district as an indication of the degree of infestation. Because districts vary so much, he used the ratio of costs in two successive years as an indication of a change in population. Changes smaller than a factor of three were disregarded. In years (July to June) when the locusts increased, there was no outstanding feature of the

current rainfall, except that dry conditions were infrequent, but the previous year's early summer (July to December) rainfall was low. Lest the rise in cost be regarded as a return to normal after a poor year, it should be noted that the two most costly years followed years with particularly poor early summer rains, and the most costly of all followed two such years in succession and occurred in a year of abnormally copious early summer rain. This relation seems to be applicable district by district. It would be interesting to see whether a multiple regression equation, relating two previous years and the current one, and including years of little change, would yield a good forecasting system.

The biological basis for these associations of rainfall and control costs is not known. Control costs do not depend simply on the number of locusts but on how many are open to attack: a given number of locusts, evenly dispersed, would not be attacked, while the same number, if in bands, would be. Whether rainfall may affect the locusts in such a way as to alter their clumpiness or to modify their numbers, remains to be investigated. *Solitaria* brown locusts tend to lay diapause eggs, which will not hatch in the season in which they are laid, while *gregaria* tend to lay nondiapause eggs, producing an immediate new generation if there is sufficient rain. These factors are apt to accentuate fluctuations in numbers of adult locusts (75).

Symmons has investigated the relation between rainfall and the sizes of red locust populations in the North Rukwa Valley outbreak area (124). Again, it is found that low rainfall in the previous year leads to a large population and again the rainfall in the current year seems to have the opposite effect; that is to say, well-distributed rain during the breeding season is beneficial. On the basis of information covering 16 years, some of it not very accurate, Symmons put forward the forecasting equation

$$F = 6.518 - 0.16R + 0.425P + 0.192r$$

where F = filial population and P = parental population, and each is expressed in five grades on a scale that is probably something like logarithmic, R = rainfall in inches (average of local and drainage basin) in the previous rain season, and r = rainfall in inches in the first three months of the breeding season that gives rise to the filial population. The total correlation coefficient was 0.93 ($p < 0.01$), and the standard error of estimate of the filial population was about half a grade. There were insufficient data to decide between possible reasons for the correlation—height of water table and readiness of soil to waterlog; effects through vegetation; effects through soil fauna; mortality of the parental generation. Although the data for the other outbreak areas were much less satisfactory, there were general indications that a similar relation held.

It is strange that rather similar forecasting principles are probably valid for the arid and semiarid zone *Locustana* (and may be found to be for some species of *Melanoplus*) and for the flood-plain *Nomadacris* (and for at least

some subspecies of *Locusta*). Considering how complex any biological situation must be, it is gratifying to find such a simple relation between long-previous weather and control required. It is scarcely to be hoped that *S. gregaria*, which is continuously swarming, sometimes migrating over an enormous area, will yield a simple correlation, for it can spread over and beyond any locally extreme conditions. In a general way, Acrididae are associated with arid or semiarid conditions and seem to be favoured by sunshine and attacked by diseases in cool and damp weather; but the eggs must take up water from the soil before they can develop (116) and food will grow only if there has been sufficient rain (131).

What is at first surprising is that since, in the outbreak areas, dry years are eventually favourable, there are not other, rather drier, outbreak areas that are more often favourable. Key, in fact, showed that in different outbreak areas of *C. terminifera*, outbreaks were favoured by certain weather variations in opposite directions and consequently no simple general forecasting system was possible (17, 59). In other cases, perhaps it is essentially the dry period within a fluctuating climate and not the dry period in isolation that is important. This is supported for both brown and red locusts by the favourable effects of rather wet conditions during the early part of the year of the big upsurge; but the continuation of wetter conditions seems to be unfavourable (61).

Schwerdtfeger regards populations as steadied by a large number of independently varying small factors (and many minor factors have been reported in different species of locusts) (114); if, however, one factor predominates, the big fluctuations observed in numbers of locusts are to be expected. The occurrence of such a dominant factor is so valuable in forecasting that it is worth searching for. If found, it should be rationally explained, lest the effect be an indirect and variable one.

CONTROL

Control of locusts is as much a matter of economics as of strategy or technique; where little damage is likely to occur, expensive measures should be avoided (5, 83). It is not easy, however, to discover the cash value of damage done by locusts and only in North America has much been done (85, 86, 134, 136, 137); little theoretical consideration has been given to it, except by Ordish (83). In the world as a whole, the estimated average annual value of damage between 1925 and 1934 was £8.3 million and of control was £1.3 million plus 9.2 million man-days of unpaid labour (133). North America accounted for an annual average of £5.9 millions of damage, which is high partly because of more complete ascertainment and partly because of a higher percentage of cultivated land. In Africa, recorded annual damage averaged £0.5 million and control £0.4 million plus 9.0 million man-days of unpaid labour. In East and Central Africa, at least, native cultivation is carried out in patches of about an acre, widely scattered in uncultivated

land, and totalling at least 10,000,000 acres. During a plague, the very men who could evaluate damage are already overworked at trying to prevent it, so damage is unrecorded. The cost to 64 countries of controlling the desert locust alone in 1956 was £5 million (95).

If control costs less than the damage it prevents, then the expenses of control plus the value of damage done can reasonably be regarded as the cost of locusts. The validity of this assumption is sometimes doubted, but people who have once been protected from locusts tend not to be objective about losing protection, even experimentally. Even when the national damage is slight, the losses suffered by a few individuals may be crippling, a situation which might be met by insurance. In a case in which insurance tenders were invited, no indications were given of whether control would be continued, as in fire insurance, of who would pay the premiums, and of the basis of compensation (7, 78).

How much, if anything, can be spent on control is generally decided politically, with advice from experts. It is always impossible to kill all the locusts, and it is a roughly accurate rule that the higher the mortality aimed at, the greater the cost per locust killed (diminishing returns); there is thus a level of control at which the cost just equals the value of the damage prevented and further control would be financially unprofitable. But if substantial damage occurs, the control organization is likely to lose public support; consequently the aim may be to kill as many locusts as resources allow. In addition, the locusts have to be regarded not only as themselves likely to do damage but also as providing the next generation.

Once control has been decided upon, there is at present a choice of at most four strategies: (a) outbreak-area control; (b) frontier defence, including defence in depth; (c) over-all attack; and (d) local defence of crops, which is a last resort and never entirely successful. The outstanding case of aggressive strategy using biological information is preventive or suppressive control in small outbreak areas protecting a plague area many times as large (*Locusta* and *Nomadacris* in Africa). Judging from the £2 million spent in South Africa on crop defence during the last red locust plague, the average annual cost over a long period would be £60,000; South Africa's share of the cost of outbreak suppression by the International Red Locust Control Service is now much less than this. The argument applies more strongly to *Locusta* in Africa, which has similar outbreak areas, a larger plague area, and a slightly cheaper control organization.

For the brown locust in South Africa, the costs of control plus its organization have been £1 million (1934 to 1944) and £2.3 million (1945 to 1956), so the annual average is about £0.15 million. Of this, the annual cost of the permanent locust-control organization has been £0.03 to £0.05 million and the remainder has been spent on control campaigns. Although the outbreak areas are 40 per cent of the size of the plague area, outbreak suppression is established practice and is reckoned to be profitable (67).

The Australian plague locust normally migrates 50 to 100 miles and not more than 500 miles (58, 63). In the State of South Australia, swarm damage is rare and slight, and it is considered wise to deal with an invasion after it arrives (5, 6); in the State of Victoria, invasions have largely been dealt with along a defensible frontier by aircraft attack (15, 50); but in New South Wales, which contains many of the extensive outbreak areas, a strategy of control is being investigated by interstate co-operation (5, 6).

With desert locusts, persistent outbreak areas are unknown but migration routes are known, so frontier defence has been considered. The frontiers to be defended are ecological and topographical but cut across political boundaries, which causes great difficulties. Unfortunately, invasion routes are in some cases very broad and invading swarms thin, so the strategy has been to kill the locusts wherever they could be attacked, especially outside crop areas (36, 98). This is bound to be costly in such a wide-ranging species, and a more sharply-pointed strategy is needed. If a desert locust plague is carried through a recession by a few swarms and is not regenerated from scattered locusts, then the destruction of those few swarms, even at considerable cost, would prevent plagues and would constitute a new strategy (see p. 282). The numbers of desert locusts at other times have usually been too great for the maximum effort made against them (97).

Until fairly recently, whatever the strategy, all attacks on migratory locusts had to be made on the nymphs (hoppers), though nonmigratory grasshoppers could be successfully attacked as adults. The use of aircraft now permits the mobility of the locusts to be exceeded. Aircraft spraying and reconnaissance have been logically and quantitatively developed in Africa (39, 40, 41, 68, 69, 93, 95, 98, 99). Dusts were soon abandoned as too unreliable. The physics of spraying, both air-to-ground and air-to-air, was worked out at the Chemical Defence Experimental Establishment, Porton, England (99, 109, 145). For attacking desert locusts in flight, the original design was to maintain a curtain of spray across their flight course by repeated aircraft runs; unfortunately the locusts do not fly parallel to one another and to the course of the swarm as a whole, as the theory demanded, and because of settling (rolling), swarms often progress more slowly than the wind. The structure and cohesion of such swarms can be explained in terms of atmospheric turbulence and of a strong tendency for locusts that reach the edge of the swarm to fly inward again. The movements of groups of locusts within a swarm are effectively random and the direction of displacement of the swarm as a whole is determined by the wind direction, which also carries the spray (96, 103, 110, 143). So another approach was needed. In a spray run across a flying swarm, many locusts would receive sublethal doses and would then mix up with undosed locusts. Accordingly, the method adopted against very large swarms of desert locusts is to treat the swarm as a whole, spraying always across the densest parts, and steadily building up lethal doses, if necessary for days, on the locusts that remain in flight. A solution of 11 per

cent γ -BHC is used because, like Diazinon, it has a cumulative action, whereas the quicker-acting 20 per cent dinitrocresol appears to be detoxicated quite rapidly, when not at once fatal (72, 74). Development is in progress of a more concentrated, less volatile, insecticide, so as to get from each aircraft load the greatest possible number of small drops which will hang long in the air.

In air-to-ground spraying, there has been a similar development through mathematical accuracy to practical field methods. The Porton technique of drop formation required rather fast aircraft, which could then spray swaths 88 to 200 yd. wide; but flexibility of operation and the difficulty of obtaining fast aircraft equipped for spraying require the use of small planes, which are too slow for the Porton technique, so boom and nozzles came into use and may soon be replaced by spinning-cage methods (11, 49). Moreover, although the use of a ground party to mark runs increases effectiveness, it greatly increases cost (68). For settled desert locusts, the tactics are to spray over the densest part of the swarm a series of separate runs, which should be lethal without overlap, and to attack the survivors later when they re-form (95). Greatly improved tactical efficiency has resulted against both desert and red locusts, and the methods of calculation and analysis originating at Porton remain the basis for development.

Improved tactics against desert locusts have made attacks on adults at least as effective as on nymphs (95, 98). Physical methods of attacking nymphs (beating, burning, trenching) have been abandoned except in remote places where labour is free. The original chemical method of hand spraying arsenical solution on the nymphs and the grass was effective, but it could be dangerous to the grass and to cattle. In 1885, the arsenic was incorporated in bait, usually wheat bran with various additions such as water, molasses, and banana oil (amyl acetate), which could not hurt grass or cattle but might not be eaten by the nymphs. No constant improvement on mortality of 70 to 80 per cent was achieved by many investigations in North America. Savings were then made without loss of mortality by omitting the additional substances, including the water, and dry bran mixed with fine BHC dust is now the standard bait for use against desert locust nymphs (42, 85), but the transport cost of bait is against its use. Today, with the new organic insecticides, methods have turned full circle and chemicals like dieldrin that are relatively harmless to stock and to plants are sprayed or drifted onto the natural food (68, 111).

With a sedentary population, there seems no alternative to treating the whole area infested; but with nymphs that migrate, sparse lines of bait or contamination of vegetation with a persistent stomach poison can be extremely economical. The greatest tactical success can be achieved with a square lattice of sprayed strips, in which a red locust infestation in bands too small for individual attack can be almost cleared if only 10 per cent of the area is sprayed with dieldrin (9, 28, 68, 69, 111, 149).

Policy, strategy, tactics, and techniques are mutually dependent and all depend on the biology of the species concerned. Close collaboration between various sorts of experts is required. In planning control, use is made not only of forecasts of numbers and of behaviour, but also of empirical information, much of which would be worth investigation and codification. Abundant opportunities remain for improvements.

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MECHANISMS OF RESISTANCE AGAINST INSECTICIDES^{1,2}

By A. W. A. BROWN

Department of Zoology, University of Western Ontario, London, Canada

It is now five years since Chadwick (50) discussed the various types of physiological mechanisms under the headings of behaviour, structure, penetration, storage, excretion, detoxication, and decreased sensitivity. Subsequent events have shown that resistance may be specific to different insecticide groups; the distinctness of these differing resistances was emphasized by Metcalf (139). Of the six types discussed in this review, the most important are DDT-resistance, dieldrin-resistance and organophosphorus (OP)-resistance, and in two of these the principal mechanism evidently is detoxication. Insect detoxication mechanisms have been reviewed by Smith (193), who distinguished 13 of them. Nonspecific resistance to various insecticides, or vigor tolerance in general, has been characterized by Hoskins & Gordon (100).

The present review builds on the previous summaries of insect toxicology contributed to the *Annual Review of Entomology* by Kearns (107), Dahm (62), and Winteringham & Lewis (216). Material on DDT-resistance has been assembled by Kearns (106) and Perry (170), on chlorinated hydrocarbons by Winteringham & Barnes (213), and on organophosphorus compounds by Fukuto (84) and Spencer & O'Brien (195). The genetics of resistance has been reviewed by Crow (61) and by Milani (145). Recent general reviews on resistance by Wiesmann (208, 209), Busvine (42), and Brown (34 to 37) have physiological content and useful bibliographies. Because of the importance of finding a direct solution of the resistance problem, the present review includes a section on negatively correlated insecticides.

DDT-RESISTANCE

DDT-dehydrochlorinase.—After the assay of many house fly strains for DDT-dehydrochlorinase activity, and initial concentration of the enzyme by Sternburg, Kearns & Moorefield (199) a method of concentrating it 200 times by ammonium sulfate precipitation or by freezing and thawing was described by Moorefield (150). Using precipitation and absorption methods, Lipke & Kearns (121) have purified it 46,000 times and found it to be a simple protein of mol. wt. 36,000 and isoelectric pt. 6.5. Although the turnover value of the enzyme is low, at equilibrium it has converted nearly all the DDT to DDE.

¹ The survey of the literature pertaining to this review was concluded in May, 1959.

² The following abbreviations are used in this chapter: BHC for 1,2,3,4,5,6-hexachlorocyclohexane; DDE for 1,1-dichloro-2,2-bis(*p*-chlorophenylethylene); DDT for 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; DFP diethyl fluorophosphate; GSH for reduced glutathione; OP for organophosphorus; SH for sulhydryl.

Lipke and co-workers (123) discovered that egg-yolk lipoprotein solubilizes DDT and facilitates the action of DDT-dehydrochlorinase. Lipke & Kearns (122) found the enzyme to be activated only by glutathione or its cysteinylglycine moiety, and to be not inhibited by SH-binding metallic ions nor by metal-binding agents such as azide. They noted its similarity in activation, solubility, and absorption characteristics with the nitroglycerin reductase of pig liver (97). The purified enzyme dehydrochlorinated DDD (TDE) [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane] four times as fast as DDT, while methoxychlor was destroyed more slowly and the unsubstituted diphenyl analogue not at all. Reuter and co-workers (185) found that the DDT analogues synergized by the *p,p*-dichlorophenyl synergists were those susceptible to DDT-dehydrochlorinase.

Lovell & Kearns (126) assayed the DDT-dehydrochlorinase of individual flies in a resistant strain and found a compact frequency distribution except for a few plus-variants. On crossing with a susceptible strain without DDT-dehydrochlorinase, they produced F_1 hybrids with about half as much enzyme as their resistant parents. The segregation ratios of individuals lacking dehydrochlorinase in the F_2 and the offspring of an $F_1 \times$ susceptible backcross were consistent with monofactorial inheritance of the enzyme. The single gene allele for DDT-resistance, clearly demonstrated by Lichtwardt (119) in Illinois, had already been discovered in Italian flies by Milani (144), who named it *kdr*. Milani & Travaglino (146) found it to be linked with the genes *bwb* and *dv*, but at a large crossover distance. Recently, Milani (147) has found the DDT-resistant gene in the Orlando-R strain to be different from *kdr* but in the same linkage group, and rendered dominant by the same genetic modifier.

Dehydrochlorination and larval life.—Moorefield & Kearns (152) traced the synthesis of DDT-dehydrochlorinase during larval life in resistant strains and found that one-half disappeared at pupation. Within a strain, those that pupated last contained 40 per cent more enzyme than the first to pupate. Moorefield (151) discovered in a susceptible strain that the larvae contained considerable DDT-dehydrochlorinase, but the adults lacked it. Kerr and co-workers (110) induced a normal Canberra strain to develop as much adult DDT-dehydrochlorinase as a parallel DDT-selected strain by selecting the late pupaters. The only difference from other strains was that it had a longer larval period. Reiff (183) examined those few flies in a normal strain which survived the first applications of DDT and found that they showed the DDT metabolism and increased lipid content characteristic of resistant strains.

Hadaway (91) demonstrated DDT detoxication in normal flies by showing that daily sublethal doses were only half cumulative. Menn, Benjamini & Hoskins (137) compared the percentage conversion to DDE of identical DDT dosages by larvae and adults and found it to be 30 and 15 for susceptible, 90 and 67 for resistant, strains. They demonstrated that higher temperature favoured recovery because the Q_{10} for detoxication was con-

siderably higher than for absorption. Although resistant strains are not characterized by reduced cuticular absorption (175), Perry (170) reported a strain submitted to DDT-DMC (Dimite; 4,4'-dichloro- α -methylbenzhydrol) pressure which progressively absorbed less DDT to the point that it became resistant to the synergized mixture.

McKenzie & Hoskins (128) also had selected the late pupating flies and obtained increases in DDT tolerance for both a resistant and a susceptible strain, with concomitant increases in larval period. They suggested that the greater susceptibility of the faster-growing individuals could explain the reversion of resistance in laboratory cultures and possibly in field populations. Indeed, Boggild & Keilding (21) found that a DDT-resistant subcolony could overcome the parent susceptible strain in crowded larval cultures because they developed slightly faster.

Mode of action of DDT.—Pant & Dahm (169) have studied the effect of DDT on houseflies *in vivo* and found that although there was no change in the cytochrome oxidase, the succinic dehydrogenase activity was inhibited. Barsa & Ludwig (14) studied the effect of DDT on homogenates of *Tenebrio* and *Musca* and discovered that succinic dehydrogenase was the only one of the nine dehydrogenases tested to be inhibited by this insecticide.

The lipoid barrier.—Wiesmann (209) found that his DDT-resistant strains at Basel were characterized by a greater content of lipoid, which was more unsaturated and could dissolve more DDT than the normal. The cholesterol content of nervous tissue was increased, and the permeability of nerve and epidermal cells for vital dyes was reduced. Injections of lipase made the resistant strains more susceptible, and feeding or injecting fat made the normal strains more resistant; the latter result had also been observed by Mer & Furmanska (138). Later, Wiesmann (210) reported that the tarsal epicuticle of resistant flies contained more lipoid than normal, and found conspicuous vacuoles in their hypodermal cells. Reiff (182) confirmed that the lipoid content of tarsi and of ganglia was higher in DDT-resistant flies, and even greater in strains made resistant to BHC, dieldrin, or Diazinon.

Recognizing their importance in taking up residual DDT in practice, Reiff (181) examined the tarsi and found that their lipoprotein became dissociated on contact with the deposit. Wiesmann & Reiff (211) discovered that the ability of the tarsal lipoid to dissolve DDT changed during the period of contact, increasing in resistant flies but decreasing in susceptible flies; on the other hand, the susceptible flies secreted twice as much tarsal grease and thus took up twice as much DDT. Reiff (182) examined those normal flies which were knocked down first and found in their tarsi the highest content of free lipoid and free protein. On the other hand, he found (184) that multiresistant flies were more able to preserve a stable condition in the lipoprotein constituents on tarsal contact with DDT, Diazinon or a carbamate insecticide. Reiff (183) had shown that DDT-susceptible flies

suffered a reduction in the amino acid level of the haemolymph. Corrigan & Kearns (59) reported that a characteristic effect of DDT on *Periplaneta* was to reduce the haemolymph level of proline.

DDT-sensitivity of nerve.—Wiesmann (207) observed that resistant flies could withstand to a considerable degree the effect of DDT in destroying the tarsal-proboscis response to sugars and in inducing autotremor of isolated legs. The isolated nerve of resistant flies was shown by Weiant (206) to be less sensitive to the action of DDT in producing bursts of action potentials. Smyth & Roys (194) found that minute doses of DDT that increased the tarsal sensitivity of normal flies to sugars and to mechanical contact did not change the threshold for resistant flies. Evidence that the DDT-resistance of nerve could be caused by detoxication was obtained by Miyake, Kearns & Lipke (149), who found resistant house fly brains to be very rich in DDT-dehydrochlorinase (second only to the fat body); they suggest that the high content of lipoprotein in nervous tissue facilitates the action of this enzyme so that the local dehydrochlorination is complete. Wiesmann & Reiff (211), however, reported that resistant flies differed only slightly from susceptible flies in DDE production *in vivo*, while Reiff (180) found even smaller differences *in vitro*, although he evidently did not use glutathione activator.

Chemical content.—Micks & Singh (143) could discern no significant difference in lipid content between several resistant and susceptible strains; nor could the interstrain differences in quality of lipid, as revealed by infrared spectrography, be correlated with resistance. March & Lewallen (132) found that the interstrain differences of materials separated by paper chromatography and detected by ultraviolet light or ninhydrin were related to the geographic origin of the strains and not to their susceptibility level. Cotty & Henry (60) concluded that the glutathione levels were similar in one susceptible and one resistant strain, but that the turnover of the cysteine in the susceptible was slower in the resistant strain. Ringel (186) found that the Orlando-Beltsville strain showed more copper than a normal one in four-day adults, but this difference was far exceeded by the sexual preponderance of copper in males of either strain.

Enzyme content.—Rockstein & Inashima (189) had reported that the Orlando strain contains more alkaline phosphatase than a normal strain, with no difference in the acid phosphatase. Alexander, Barker & Babers (1) found no difference in alkaline phosphatase between this strain and three normal strains; however, there was slightly more acid phosphatase in the resistant females than the normal females, but the sexual preponderance of this enzyme in females over males was far greater. Frontali (83) compared the cholinesterase activities of nine resistant with six susceptible strains, and discerned no consistent difference between the two groups.

Periplaneta.—The rapid absorption, metabolism, and excretion of radioactive DDT by *Periplaneta americana* (Linnaeus) has been demonstrated by Robbins & Dahm (187), who found that the excreta contained about 5 per cent DDT, 4 per cent DDE, while 11 per cent comprised three minor

metabolites, and nearly 80 per cent comprised two major metabolites that were diphenyl-2-carbon derivatives but not DDA [*bis-(p-chlorophenyl)* acetic acid]. Butts & co-workers (47) had reported water-soluble metabolites that could be rendered ether-soluble by acid refluxing, which they considered to be conjugation products. Hoskins & Witt (101) confirmed that the American roach excretes some DDT and DDE, along with oxidation products, one of which chromatographs close to dichlorobenzophenone. Hoskins & co-workers (103) have recently reported that two of these metabolites simulate DDE in the Schechter-Haller reaction.

Although DDT-resistance has never been demonstrated in *Periplaneta* it has been suspected in South America (34), and there is a marked sexual difference in DDT tolerance. Females are three to five times more tolerant than males, even when allowance is made for their greater body weight, not only for DDT (124) but also for other chlorinated hydrocarbons (54) and even inorganic insecticides (96). Munson & Gottlieb (156) found that females contained 10 to 20 per cent more total lipid per unit body weight than males; while Lofgren & Cutkomp (124) established a correlation between lipid content and DDT tolerance for individuals within either sex. However, the difference in lipid content was considered insufficient to account for the sexual difference in the size of the fat body, which Forgash (81) found to be eight times heavier in the female than the male. It was the fat body which, of all tissues assayed by Cochran (55), contained the highest content of administered DDT and of metabolites which gave the Schechter-Haller reaction for DDE.

Blattella.—The metabolites produced from topically applied radioactive DDT by a fairly susceptible strain of the German roach have been studied by Hoskins, Erwin & Andrews (102), who found virtually no DDE but instead four metabolites more polar than DDT. The least polar product, Metabolite 4, occurred in the tissues and not the excreta; on alkali refluxing it released dichlorobenzophenone. The two most polar products proved on greater chromatographic resolution to be really five metabolites.

DDT-resistance has not developed in field populations of *Blattella germanica* (Linnaeus) because chlordane has been applied as the more effective insecticide. However, laboratory pressure with DDT has produced a strain in which the males are 20 times and the females 200 times more resistant than normal (89). This DDT-resistance is matroclinous in its inheritance, and has been found by Grayson (90) to revert after several generations of release from pressure. Hoskins, Miskus & Eldefrawi (103) demonstrated that this resistant strain metabolized DDT at a greater rate than the susceptible strain, to the same spectrum of metabolites. They point out that the DDE reported by Babers & Roan (11) to have been produced in small amounts by susceptible *Blattella* and in slightly larger amounts by a chlordane-resistant strain was probably a mixture of Metabolites 3 and 4.

Other Orthoptera.—The somewhat DDT-tolerant Madeira roach, *Leucophaea maderae* (Fabricius), was found by Lindquist & Dahm (120) to absorb

DDT comparatively slowly and then to excrete within three days 19 per cent DDT, 4 per cent DDE, 1 per cent Metabolite A, 13 per cent Metabolite B and 51 per cent Metabolite C. Lindquist & Dahm consider the last two metabolites to be conjugates of DDE and DDT, respectively, and emphasize their similarity to the two major metabolites in *Periplaneta*. The highly DDT-tolerant *Melanoplus* grasshoppers had been found by Sternburg & Kearns (197) to absorb very little DDT through cuticle or stomach, and to convert it rapidly to DDE, which was further metabolized by *Melanoplus femur-rubrum* (DeGeer) but not by *Melanoplus differentialis* (Thomas).

Pediculus.—DDT-resistance of the body louse (*Pediculus humanus humanus* Linnaeus) has developed in several parts of Asia, Africa, and South America (220). It extends to methoxychlor (73) and DANP (dianisyl-neopentane) (40), but not to *o,p'*-DDT (223). It may be countered by adding the synergist piperonylcyclonene (224) but not by adding Dimite (DMC) to DDT (57).

Kearns (106) reported that the Korean resistant strain could metabolize topically applied DDT to a material which gave the Schechter-Haller reaction for DDE. Perry & Buckner (173) found that this strain, and normal body lice also, absorbed an insignificant amount of DDT through the cuticle. With ingested DDT, Korean body lice could resist 10 times the normal dose by metabolizing it to a polar conjugate whose nitration product resembled that of *p*-chlorobenzoic acid in that it was alkali-soluble and required acidification to show the Schechter-Haller reaction for DDE. When incubated with glutathione, homogenates of both susceptible and resistant strains *in vitro* converted DDT to this water-soluble metabolite. Perry & Buckner suggested that this detoxication is inhibited in normal body lice *in vivo*.

Perry (171) proceeded to purify the enzyme involved and found that it was activated by glutathione, cysteine or ascorbic acid, and that its optimum pH was 8 to 10. This DDT-ase was remarkable in not being precipitated by ethanol nor hydrolyzed by proteases and in remaining active after being boiled for an hour.

Caterpillars.—A typical example of this DDT-susceptible group is the cabbage cutworm, *Barathra brassicae* Linnaeus, which Kojima and co-workers (112) found to dehydrochlorinate only 3 per cent of the absorbed DDT, whereas a DDT-resistant strain of *Pieris rapae crucivora* Boisduval converted more than 80 per cent to DDE in the same period. Lindquist & Dahm (120) discovered that mature larvae of *Pyrausta nubilalis* (Hübner), which are somewhat DDT-tolerant, produced DDE as the only metabolite and accumulated as much DDE as DDT by the fifth day after treatment.

The normally DDT-tolerant red-banded leaf roller *Argyrotaenia velutinana* (Walker) was found by Sternburg & Kearns (197) to absorb DDT through the integument and stomach so slowly and to convert it to DDE so rapidly that no DDT is present in the body. In contrast, DDD (TDE) [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane], to which the leaf roller is

susceptible, is not metabolized rapidly and only a trace of the dehydrochlorinated DDD is found. DDD-resistant strains of *Argyrotaenia* have now developed in New York State (88). The tobacco hornworm *Protoparce sexta* (Johanssen) is normally much more DDT-tolerant than its congener *P. quinquemaculata* (Haworth). Hoskins & Witt (101) report that *P. sexta* produces large amounts of DDE from DDT, but is susceptible to DDD, which it cannot dehydrochlorinate. DDT-dehydrochlorinase has been demonstrated for this species in tissues homogenized in glutathione.

Hoskins & Witt (101) have studied other susceptible caterpillars. Larvae of *Malacosoma americanum* (Fabricius) rapidly produced DDE, but absorbed DDT so fast that it was still susceptible. Larvae of *Bombyx mori* (Linnaeus) produced little DDE and no other metabolites; larvae of *Nymphalis antiopa* (Linnaeus) produced even less DDE but much of other metabolites; larvae of *Plodia interpunctella* (Hübner) produced much DDE and even more of other metabolites.

Coleoptera.—The normally DDT-tolerant larvae of the Mexican bean beetle, *Epilachna varivestis* Mulsant, were found by Sternburg & Kearns (197) to dehydrochlorinate DDT rapidly to DDE, which was subsequently metabolized; only 30 per cent of the contact dose was absorbed in one day, and of this only 10 per cent remained as DDT. Chatteraj & Kearns (53) have homogenized the larvae in glutathione or thiomalate and demonstrated a DDT-dehydrochlorinase which is active also on DDD and methoxychlor. Adults of the normally DDT-tolerant boll-weevil, *Anthonomus grandis* Boheman, were found by Blum, Earle & Roussel (18) to absorb about half a contact dose of DDT in one day and to convert two-thirds of it to unknown metabolites. Small amounts of DDE were produced, but this compound was not further metabolized. An endrin-resistant strain which had a slight cross-tolerance to DDT accumulated slightly less and metabolized slightly more of this insecticide.

The normally DDT-tolerant milkweed bug, *Oncopeltus fasciatus* (Dallas) was originally reported by Ferguson & Kearns (77) rapidly to metabolize DDT to unidentified products but not to DDE. Hoskins & Witt (101) detected "perhaps a little DDE," a more polar metabolite which was not DDA, and a significant accumulation of DDT in this species. The somewhat DDT-tolerant soft tick, *Ornithodoros coriaceus* Koch, they found to metabolize DDT almost completely to metabolites that did not give the Schechter-Haller reaction. A DDT-resistant strain of the cattle tick, *Boophilus microplus* (Canestrini), studied by Roulston (190) also produced no DDE and contained no DDT-dehydrochlorinase.

Drosophila.—The DDT-resistance developed in adult *Drosophila melanogaster* Meigen by laboratory selection extends also to BHC and other insecticides; Crow (61) attributed such nonspecific resistance to multiple genetic factors and not to dehydrochlorination of DDT to DDE; thus he considered it largely vigor tolerance. Yet Bochnig (19) found that the respiratory rate of a DDT-resistant Berlin strain remained at the normal level

in the males but was lower than normal in the females. Lüers and co-workers (127) reported that this strain showed the nerve histopathology consequent on DDT intoxication less frequently than a normal strain. Bochnig (20) found also that three DDT-tolerant strains were all about 15 per cent larger than normal in head, wing, and weight, consequent upon a longer larval period. Hunter (105) was, however, unable to increase the DDT-tolerance of a mixed stock of normal *Drosophila* by selecting for a long larval period, but he could increase its DDT-susceptibility by selecting the early pupaters.

The high DDT-resistance developed in Japanese strains by larval selection was again attributed, by Tsukamoto, Ogaki & Kikkawa (205), to a gene at locus 66 on the second chromosome, and it was found to extend to BHC, parathion, and other insecticides. Oshima (167) concluded from a study of three strains that DDT-resistance could be derived from polygenes but that the highest resistance was given by the gene on the second chromosome.

Aedes.—Brown & Perry (33) found that larvae of the DDT-resistant strain of *Aedes aegypti* (Linnaeus) from Trinidad converted much of the DDT to DDE, whereas a susceptible laboratory strain did not convert the much lower dose applied. Subsequent experiments (52) at identical DDT concentrations showed that the Trinidad strain produced much DDE and an inbred laboratory strain almost none, while a susceptible Malayan strain, which subsequently proved to have a high resistance potential, produced considerable DDE. Brown (32) incubated homogenates of the Trinidad strain with glutathione but no DDT-dehydrochlorinase activity appeared. Busvine & Coker (45) found that Dimite (DMC), which inhibits this enzyme in house flies, was not synergistic with DDT for resistant larvae; but Fay and co-workers [vide (34)] demonstrated a degree of synergism in the adults of the Trinidad strain. The inheritance of DDT-resistance in this strain was found by Coker (56) and by Qutubuddin (178) to be explicable by a single gene allele.

Gjullin, Lindquist & Butts (87) had demonstrated that both DDT-resistant and normal larvae of *Aedes nigromaculis* (Ludlow) were active in converting DDT to nontoxic metabolites; the resistant strain actually absorbed DDT at a greater rate. Brown & Perry (33) found that resistant *Aedes taeniorhynchus* (Wiedemann) larvae produced much DDE, the susceptible larvae virtually none; however, DDT-dehydrochlorinase could not be demonstrated in either strain (32).

Culex.—Bami, Sharma & Kalra (12) discovered that normal adult *Culex fatigans* Wiedemann converted 40 per cent of contact-applied DDT to DDE, while resistant adults converted 60 per cent during one day; there was little evidence of other metabolites. Hoskins, Miskus & Eldefrawi (103) found that both susceptible and resistant larvae of this species (*Culex quinquefasciatus* Say) produced DDE; but the resistant strain absorbed more DDT, converted nearly all of it to DDE, and subsequently excreted large

amounts of two polar metabolites. Perry (172) reported that DDT-resistant adults of *Culex pipiens molestus* Forskål took up large amounts of residual DDT but converted less than 20 per cent to DDE in one day.

Anopheles.—DDT-resistance is now known in four species of anophelines (34). Gartrell & Ludvik (85) reported that larvae and adults of *Anopheles quadrimaculatus* Say collected from treated areas in Alabama produced considerably more DDE than those from untreated areas. Perry (172) found that DDT-resistant adults of *Anopheles sacharovi* Favre exposed to DDT residues contained a DDE/DDT ratio of 120 to 150 per cent 24 hr. later; a highly resistant strain from Adana, Turkey, could withstand a much higher dosage and thus produce much more DDE than a slightly resistant strain from Skala, Greece. This ratio was 30 to 45 per cent in comparatively susceptible Italian populations of *Anopheles maculipennis* Meigen, *Anopheles labranchiae* Falleroni, *Anopheles claviger* (Meigen), and *Anopheles atroparvus* van Thiel. Laboratory strains of *A. atroparvus* and *Anopheles stephensi* Liston in Rome showed DDE/DDT ratios of about 65 per cent; resistant laboratory strains of these two species also showed a low ratio at low doses, and even lower at the higher dosages that they could withstand. The resistant laboratory strain of *A. atroparvus*, which produced little DDE, shows some cross-resistance to dieldrin; whereas the resistance of the Adana strain of *A. sacharovi*, which produces much DDE, was found by de Zulueta (229) to be specific to DDT and not to extend to dieldrin. That the resistance of the Rome strains of *A. atroparvus* results from vigor tolerance factors is suggested by the findings of Neri, Ascher & Mosna (157) that the females contained a slightly higher lipid content than susceptible strains and by the figures of Mosna (153), showing that the resistance developed slowly without any flattening of the dosage-mortality regression line.

DDE production *in vitro* could not be demonstrated by Perry (172) in homogenates of the highly resistant *A. sacharovi*. However, Kearns (108) was able to demonstrate its presence in DDT-resistant *Anopheles sudaicus* (Rodenwaldt) from Java and its absence in a susceptible strain. DDT-resistance in *A. sudaicus* has been traced by Davidson (68) to a single recessive gene allele.

Perry (172) obtained evidence that 20 per cent of the Skala strain of *A. sacharovi* recovered from a knockdown which had foreshortened their uptake of residual DDT. Early knockdown and subsequent recovery characterizes the Rome resistant strains of *A. atroparvus*. In all these *Maculipennia* anophelines, the lower mortalities obtained by the test method of Busvine & Nash (46) as compared with the standard WHO method was found by de Zulueta (229) to result from the lower activity and DDT uptake in the smaller tube used in the former method.

Protective knockdown is one mechanism preventing the uptake of a lethal dose; another mechanism is departure from the deposit before sufficient DDT has been taken up. Trapido (203) first observed that populations of *Anopheles albimanus* Wiedemann along the Rio Chagres, Panama,

had become resistant to DDT antimalarial applications because the engorged females rested for a shorter period on the sprayed walls than formerly. Their physiological susceptibility to DDT was found by Trapido (204) to have remained normal. When two Rio Chagres populations were compared by Brown (38) with a normal population from an untreated area, their excitation times from DDT-impregnated paper proved in general to be significantly shorter than the normal, although the frequency distributions overlapped. However, there was a difference between wild-caught and laboratory-reared females when they were compared in treated huts by Duret (72). Populations of *A. sacharovi* from Greece, Italy, and Roumania were reported by de Zulueta (229) to be highly irritable to DDT deposits, and so were strains of *A. maculipennis* and *A. labranthiae* on the mainland of Italy. On the other hand, a strain of *A. albimanus* reared in the laboratory for 20 years was found to show much longer excitation times than three field strains (38).

The increase in DDT-resistance of a laboratory colony of *A. atroparvus* was discovered by D'Alessandro, Frizzi & Mariani (63, 64) to be associated with an increase in the number of heterozygotes for an inversion on chromosome III. Mosna and co-workers (154, 155) found this to be true for three other DDT-resistant laboratory colonies, and for a dieldrin-selected strain.

PROLAN-RESISTANCE

DDT-resistant house flies are characteristically susceptible to the Dilan compounds Prolan and Bulan (34, 139), except for one knockdown-resistant strain which was also not susceptible to DANP (dianisyl-neopentane), another nondehydrochlorinatable analogue of DDT (40). However, Dilan pressure on normal house flies produced a strain with strong cross-resistance to DDT, and Brown (32) discovered it to be rich in DDT-dehydrochlorinase. Perry & Buckner (174) found that the resistance of this strain was much greater to Prolan than to Bulan; it characteristically excreted Prolan (or a substance similar to it), followed by an acidic metabolite which lacked the NO_2 group.

DIELDRIN-RESISTANCE

Busvine (41) distinguished dieldrin-resistance from that to DDT on the basis of symptomatology and cross-resistance in the house fly, and he concluded that it extended to γ -BHC as well as dieldrin and other cyclodiene derivatives such as chlordane, heptachlor, aldrin, and endrin. Kearns (106) came to a similar conclusion, and found that a purely dieldrin-resistant strain lacked DDT-dehydrochlorinase. The separateness of DDT-resistance and dieldrin-resistance *sensulato* has also been demonstrated in the house fly by genetical means (13, 39). This resistance to dieldrin and γ -BHC is now known in 15 species of *Anopheles* and 35 other species of arthropods, and its incidence is separated from DDT-resistance (34). Dieldrin resistance is often so intense and develops so fast that postadaptation has been suspected.

Mode of action.—The present ignorance of resistance mechanisms for dieldrin stems from inadequate knowledge of its mode of action. Dieldrin-poisoned nerves of *Periplaneta* show spontaneous bursts of action potentials with prolonged after-discharge, and a flaccid paralysis follows their exhaustion (221). The motor neurones of the tarsi of *Blattella* or *Drosophila* exposed to residual deposits of heptachlor, chlordane, aldrin, or endrin show their exhaustion by the disappearance of the Nissl granules (188). Heptachlor-poisoned *Blaberus* exhibit a characteristic pycnosis of the globuli in the corpora pedunculata of the brain (118). That the symptoms of dieldrin intoxication in *Periplaneta* are antagonized by nicotine suggests that dieldrin affects the synapses of the central nervous system (86). It is characteristic of the cyclodiene derivatives that there is a long latent period between their application and the appearance of the nerve discharges in *Periplaneta* (117) and the neurotoxic increase in respiratory rate in *Blattella* (94).

Cyclodiene compounds are oxidized in the body; aldrin is converted to dieldrin in mammals (216) and *Periplaneta* (86); isodrin becomes endrin (216), and heptachlor becomes its epoxide (176) in the house fly. Heptachlor epoxide is probably metabolized by houseflies (176); it has been suggested that dogs hydrolyse it to a diol and then convert it to a phenol (65).

Resistance mechanisms.—Hoffman & Lindquist (98) found that the disappearance of topically applied chlordane or toxaphene in 10 Orlando-R strains was greater than the loss of applied DDT. A multiresistant strain was compared with normal house flies by March, Metcalf & Baich (134), who could discover no differences in their rates of cuticular absorption or fate of aldrin or dieldrin. Brooks (31) reported that resistant and susceptible strains absorbed isodrin and converted it to endrin at similar rates. Heptachlor-resistant flies were found by Perry, Mattson & Buckner (176) to absorb heptachlor and produce the epoxide at the same rate as normal flies. They could withstand an internal dose eight times the normal LC_{50} , but developed symptoms and mortality if subsequently starved for two days. It was therefore suggested that the resistance mechanism might be "solubilization or storage in a non-sensitive tissue." Indeed, Gianotti, Metcalf & March (86) had observed that dieldrin accumulated in the fat body of *Periplaneta*.

Yamasaki & Narahashi (222) have detected a decreased dieldrin-sensitivity in the exposed nerves of resistant houseflies, the latent period between the application of dieldrin and the appearance of discharges being 50 per cent longer than in a normal strain. Winteringham & Harrison (215) compared a dieldrin-resistant with a susceptible strain of *Musca domestica vicina* Macquart and found that they were alike in absorbing the radioactive sulfur analogue of dieldrin and metabolizing it to water-soluble products at about the same rate; they also excreted the unchanged dieldrin analogue, but the resistant flies continued to excrete after the susceptible strain had ceased to do so. Davidson (67) has shown that the dieldrin-resistance of *Anopheles gambiae* Giles in parts of West Africa is caused by a single gene allele and extends to other cyclodiene compounds and γ -BHC

(66). However, the dieldrin-resistant adults showed no quantitative or qualitative abnormalities in body lipoids (28). Bradbury & Standen (24) found that these adults absorbed γ -BHC and converted it to water-soluble metabolites at the normal rate.

BHC-RESISTANCE

The isomers of BHC were found by Bradbury (22) to be metabolized faster by the house fly than any of the six other species he tested. Gamma-BHC in daily doses was scarcely cumulative in the normal house flies tested by Hadaway (91), whereas dieldrin was about two-thirds cumulative. BHC-resistant strains from Denmark were found by Oppenoorth (163) to metabolize γ -BHC more than twice as fast as a normal strain, and the alpha and delta isomers almost twice as fast (164). In comparing eight different strains, Oppenoorth discovered that their BHC-resistance levels were directly proportional to the rate at which they metabolized the alpha and delta isomers; they were also inversely proportional to the rate at which they were absorbed through the cuticle, the maximum difference being of the order of three times (165).

Bradbury & Standen (23) found that a BHC-resistant strain from Uruguay converted γ -BHC to water-soluble metabolites slightly more rapidly than a susceptible laboratory strain. Moreover, the resistant flies absorbed radioactive γ -BHC or α -BHC vapour more slowly, so that the internal content was only one-quarter that in the susceptible flies (25). Bradbury (22) detected 11 different water-soluble metabolites of the radioactive gamma or alpha isomers, but CO_2 was not one of them. Alkaline hydrolysis of these metabolites has been found by Bradbury & Standen (27) to yield 61 to 68 per cent of dichlorothiophenols. They induced homogenates and enzyme preparations of flies to produce dichlorothiophenols from either BHC isomer *in vitro* by adding glutathione as an activator.

Sternburg & Kearns (198) studied an Illinois resistant strain which metabolized γ -BHC about four times as fast as a normal strain, there being no difference in the cuticular absorption rates. Lacking DDT-dehydrochlorinase, the strain produced pentachlorocyclohexene, the initial dehydrochlorination product of BHC. The pentachlorocyclohexene was evidently further metabolized, since its level in the fly remained constant at about 12 per cent of the dose while γ -BHC steadily disappeared. Bradbury & Standen (26) found that in the Uruguay strain the CCl_4 -soluble fraction, which would include trichlorobenzene and acidic metabolites as well as pentachlorocyclohexene, constituted less than 3 per cent of the dose.

Bridges (30) compared a BHC-resistant and a susceptible strain that absorbed γ -BHC and excreted its water-soluble metabolites at the same rate and discovered no difference in total lipid content. Adults of the resistant strain survived internal concentrations of insecticide far in excess of those which killed the susceptible strain. When Bridges applied microcrystals of γ -BHC to the surface of the thoracic ganglion, toxic symptoms developed in

the normal flies but not in the resistant flies. When γ -BHC was applied to the thoracic ganglion in Ringer's solution, Yamasaki & Narahashi (222) found that the discharges appeared later in a resistant strain than in normal house flies.

ORGANOPHOSPHORUS-RESISTANCE

Resistance to organophosphorus (OP) insecticides has developed in 11 species of orchard mites, six species of aphids, and seven species of Diptera (37). OP-resistance was first reported for the house fly in 1955; it is a separate entity in that DDT-resistance or dieldrin-resistance does not extend to OP compounds (139). The resistance includes all OP insecticides in greater or less degree, but it is usually greatest to the particular compound used as the pressure agent. Busvine (44) found that field strains from Italy and Denmark were resistant to Diazinon and parathion but not to malathion, whereas the reverse was true of a strain from Florida; all were resistant to Chlorthion and dicapthion. A malathion-resistant strain from a malathion-treated dairy in Florida was found by Labrecque & Wilson (116) to remain susceptible to parathion and to Dipterex (*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate). Conversely, a Diazinon- [*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-primidinyl) phosphorothiate] resistant strain from a Diazinon-treated barn in New Jersey was found by Forgash & Hansens (82) to remain only slightly tolerant of malathion, yet resistant to parathion, Chlorthion [*O*-(3-chloro-4-nitrophenyl)*O,O*-dimethyl phosphorothioate], and ronnel [*O,O*-dimethyl-*O*-(2,4,5-trichlorophenyl) phosphorothioate]; they suggest that the detoxication mechanism for this phosphorodithioate is different from that for the four phosphorothioates mentioned. Nevertheless, this strain had been found by Hansens (92) to show some malathion-resistance on residual deposits. Field strains in Florida (116a), Georgia, and Arizona (192), where different organophosphorus compounds have been applied, now show resistance to malathion, Diazinon, parathion, and Dipterex.

Recent reviews by Casida (48), Fukuto (84), and Metcalf (140) have described in detail the metabolic processes whereby OP insecticides are initially oxidized to more potent cholinesterase (ChE) inhibitors, e.g., $P=S$ to $P=O$ to produce malaoxon and para-oxon. An exception is Dipterex, which may be converted to the active inhibitor DDVP (2,2-dichlorovinyl dimethyl phosphate) by a dehydrochlorinative rearrangement which Metcalf, Fukuto & March (141) found to take place in house-fly tissues. In the review by Spencer & O'Brien (195), it is generally accepted that the primary action of OP compounds is the inhibition of ChE. Subsequently van Asperen (9) pointed out that DDVP or parathion at knockdown doses inhibited the aliesterases much more than the ChE; but recently Stegwee (196) has found that almost complete inhibition of aliesterases by tri-*o*-cresyl phosphate, in a dose that spares the ChE, leaves the house fly perfectly normal.

The possibility that OP-resistant house flies contained more ChE was first investigated by Chadwick (49), but he found his DFP-resistant strain

to contain the normal amount. March (130) did not find any consistent difference in ChE between four OP-resistant and four OP-susceptible strains, nor could van Asperen & Oppenoorth (10) between three OP-resistant and four OP-susceptible strains. Quarterman (177) reported no consistent differences in ChE among the strains at the Savannah laboratories. Furthermore, neither March (130) nor Oppenoorth (166) found any evidence that the ChE from resistant strains was less OP-sensitive *in vitro* than the normal.

In vivo, however, a malathion-resistant strain from Savannah showed over 20 per cent ChE inhibition to topical doses of malathion that caused 90 per cent inhibition in the NAIDM (National Association of Insecticide and Disinfectant Manufacturers) strain (201). Thus it was found by Pal (168) that ACh accumulated in the normal house flies but not in this resistant strain. That the cuticular barrier might be a factor was at first suggested by the finding of Busvine (42), with the DFP-resistant strain, that the OP-resistance disappeared when the dose was injected. However, both March (130) and Oppenoorth (166), working with more highly resistant strains, reported that the parathion and malathion resistance was still present when the insecticides were injected.

Oppenoorth (166) discovered a higher detoxication rate in OP-resistant house flies. The relative susceptibilities of a normal, a slightly tolerant, and a resistant strain to injected parathion paralleled the extent to which para-oxon accumulated in the body. This did not result from differences in toxifying rates, since the relative susceptibilities to contact para-oxon were the same as to contact parathion. The differences were correlated in inverse ratio to the rate at which they metabolized para-oxon. This detoxication, as Metcalf (140) has pointed out, could be partially attributed to alkaline hydrolysis, yielding nitrophenol and diethyl phosphoric acid. It could also be catalysed by a para-oxon-insensitive aromatic esterase; such an A-esterase has been found by Metcalf and co-workers (142) to be abundant in honey-bee abdomens and *Periplaneta* guts and to hydrolyse para-oxon about half as readily as phenyl acetate. Winton, Metcalf & Fukuto (218) developed the idea of protective detoxication by showing histochemically that phenyl thioacetate could be hydrolysed by the nerve cord of house flies. Contrary evidence was reported by Lord & Solly (125): DFP-resistant house flies metabolized no more para-oxon than normal, and housefly A-esterase preparations did not hydrolyse para-oxon.

Further support for the role of detoxication in OP-resistance was obtained by March (131) with a highly malathion-resistant strain of flies which he found to metabolize malaaxon twice as fast as a normal strain. There was no difference in the toxifying rate, the relative susceptibilities to contact malaaxon being the same as to contact malathion. Detoxication of malathion is principally carried out by the fat body, as indicated by the experiments of O'Brien (159) on homogenates of *Periplaneta*. The degradation is hydrolytic, and is caused either by carboxyesterases removing one or both of the ethyl esters of the maleic acid moiety or by phosphatases splitting off dimethyl

thiophosphate or phosphate; Krueger & O'Brien (113) found the latter process to predominate in the normal house fly and identified seven of the products. The toxicity of malathion to house flies may be slightly reduced, as Rai & Roan (179) have found, by prior treatment with piperonyl butoxide.

A Diazinon-resistant strain of house flies from New Jersey has been studied by Krueger, O'Brien & Dauterman (114); it absorbed topically-applied Diazinon slightly more slowly than a normal strain and came to contain slightly less diazoxon internally. When applied by contact daily, Diazinon was found by Hadaway (91) to be an almost completely cumulative poison for normal house flies.

Recently, van Asperen & Oppenoorth (10) reported a decisive difference between OP-resistant and normal house flies. Out of seven strains studied, the resistant strains always contained less aliesterases; the activity against aliphatic esters was less than a quarter, and that against aromatic esters less than a half of the normal. The association of low aliesterases with OP-resistance was demonstrated genetically throughout successive generations of backcrossing with normal flies while maintaining Diazinon pressure. A malathion-resistant strain from Savannah was also abnormally low in aliesterases. In this strain, however, histochemical tests made with butyrylthiocholine by Connell (58) have shown the incidence of aliesterases to be higher than in a normal strain.

Reiff (182) found that a Diazinon-resistant strain developed by laboratory pressure at Basel contained one-third more lipid than normal in the tarsi and thoracic ganglia; this strain also metabolized DDT faster than normal. A parathion-resistant strain developed in the Riverside laboratory was found to be highly resistant to DDT and to convert it rapidly to DDE (201). March, Lewallen & Metcalf (133) reported that their OP-resistant strains developed by pressure from various OP compounds showed an even higher cross-resistance to DDT and other chlorinated hydrocarbons. The same was found by Meltzer (136) in a strain developed by Diazinon pressure; in addition, selection with the carbamate compound S.17 (N-dimethyl phenylcarbamate) induced cross-resistance to Diazinon and to the chlorinated hydrocarbons. Eldefrawi, Miskus & Hoskins (74) discovered that their parathion-resistant strain showed a cross-resistance to Sevin, another carbamate anticholinesterase. A similar observation was made by Forgash & Hansens (81) with their Diazinon-resistant strain.

A behavioural avoidance of malathion in sugary baits has been reported by Kilpatrick & Schoof (111), who found it to be present in only one dairy in Savannah where the house flies were slightly malathion-resistant. Fay, Kilpatrick & Morris (76) submitted this Bethesda strain to malathion pressure and found that the avoidance became more pronounced as the malathion resistance increased. Subsequently this avoidance has been found elsewhere in Savannah (192) and in an Orlando malathion-resistant strain tested by Schmidt & Labrecque (191); in no case did this avoidance extend to Dipterex or DDVP (2,2-dichlorovinyl, dimethyl phosphate).

OP-resistance is even more important in the tetranychid mites than in house flies; the situation is similar in that it extends to a spectrum of OP compounds in varying degrees (140). Of the underlying biochemical mechanisms nothing is known, as March (129) has pointed out. However, Taylor & Smith (200) have studied the OP-resistance in *Tetranychus bimaculatus* Harvey and *T. cinnabarinus* Boisduval by means of diagnostic dosage of malathion and found it to be inherited as a single dominant factor. Similarly Nguy & Busvine (158) found the parathion-resistance of an Italian and a Danish strain of house flies to be attributable to one dominant gene and the malathion-resistance of an American strain to be due to another, possibly allelic with it.

PYRETHRIN-RESISTANCE

Resistance to pyrethrins has recently developed in house flies in Sweden (69) and in the German cockroach in Texas and the southeastern States (109). House flies have become more tolerant of pyrethrin aerosols throughout the United States (75). Pyrethrin-tolerant strains have been developed by laboratory pressure, and they showed cross-tolerance to chlorinated hydrocarbons (70, 93). Strains resistant to chlorinated hydrocarbons remained susceptible to pyrethrins (34), except for one exhibiting vigor tolerance to many insecticides (212) and another in which there was cross-resistance from DDT to Prolan and DANP (dianisyl-neopentane) (40). Danish populations resistant to chlorinated and OP compounds showed quite high cross-resistance to pyrethrins (69). An African population of bedbugs highly resistant to DDT and dieldrin showed a ten-fold cross-resistance to pyrethrins (43).

Some years ago, Woke (219) demonstrated that pyrethrins were detoxified by the tissues of *Prodenia* larvae, the fat body being the most active. Chamberlain (51) made lipase extracts from *Periplaneta* and found that they slowly hydrolysed pyrethrins; addition of synergists such as piperonyl butoxide inhibited this hydrolysis. Enzymic detoxication of pyrethrins by tissues of the house fly was demonstrated by Matsubara (135), who established that it was inhibited by synergists but not by related nonsynergists. The mode of action of pyrethrin synergists has been fully reviewed by Kearns (107) and by Dahm (62).

Evidence of processes other than hydrolysis was obtained by Zeid and co-workers (228) who found an unknown metabolite in the muscle and brain of pyrethrin-poisoned *Periplaneta* in addition to keto-alcohols and chrysanthemum acids. Winteringham, Harrison & Bridges (217) reported that the rapid detoxication of radioactive pyrethrins and allethrin by house flies, inhibited by piperonylcyclonene, left only keto-alcohols in the body. A similar absence of chrysanthemum acids was reported by Hopkins & Robbins (99) for the excreta from house flies treated with allethrin, which contained a substance resembling allethrolone; although nearly half of the dose was excreted in the first day, no unchanged allethrin was present. Bridges (29)

found that allethrin was rapidly absorbed and metabolized by normal house flies, the detoxication being faster in the abdomen than in the thorax and in females than in males. However, lipase preparations even from female abdomens were relatively inactive in detoxifying allethrin and were unaffected by piperonylcyclonene; it was concluded that hydrolysis plays a small part, if any, in the breakdown of allethrin.

CYANIDE-RESISTANCE

Previous ideas that the resistance of strains of *Aonidiella aurantii* (Maskell) to HCN might be explained by tracheal closure were disproved by the experiments of Yust (225). Finding that the oxygen consumption of resistant strains was much less inhibited by HCN and H₂S than normal and that resistant strains were much more susceptible to anoxia than normal, Yust & Sheldon (226) suggested that their respiration might be largely independent of cytochrome oxidase and dependent on an autoxidizable flavoprotein enzyme. Recently Kurland & Schneidermann (115) have found that the insensitivity of the diapause respiration of Saturniid pupae to HCN and CO results from a great excess of cytochrome oxidase over cytochrome-*c*.

Yust, Nelson & Busbey (227) had shown that the waxy covering of the scale was not involved in the difference of the resistant strain from the normal. By means of backcrosses of the F₁ hybrids, they demonstrated that the inheritance of HCN-resistance was referable to an incompletely dominant sex-linked factor, thus confirming the conclusion reached by Dickson (71) from crosses within the F₁ hybrids. That insects can detoxify HCN to thiocyanate was established by the work of Beran on *Gasterophilus* larvae [as reviewed by Smith (194)].

ARSENIC-RESISTANCE

Resistance has been developed by the codling moth [*Carpocapsa pomonella* (Linnaeus)] to acid lead arsenate, by the peach twig borer (*Anarsia lineatella* Zeller) to basic lead arsenate, and by the cattle ticks *Boophilus decoloratus* (Koch) and *Boophilus microplus* (Canestrini) to sodium arsenite (34, 139). It has been studied mainly in *Carpocapsa*, and here Haseman & Meffert (95) found that larvae of the resistant Colorado strain were just as susceptible to oral doses of sodium arsenite and acid lead arsenate as the susceptible Virginia strain. Hough (104) discovered that the Colorado larvae were resistant to spray deposits of all the other insecticides he tested and, in fact, were more successful than the Virginia larvae on unsprayed apples also. The difference was that the newly hatched Colorado larvae were more resistant to the loss of weight that normally occurs on starvation, and they were more resistant to HCN; he therefore concluded that "the difference seems to be one of general vigor" and found it to be limited to the embryonic and young larval stages. The inheritance of this character, as judged by reciprocal crosses and backcrosses between the Colorado and Virginia strains, was traceable to autosomal factors without dominance.

The effect of arsenicals on insects resembles that on mammals in that they combine with glutathione, as demonstrated by Fink (78) in 10 different species treated with As_2O_3 and As_2O_5 . Forgash (79) found that the toxicity of As_2O_3 and Na_2HAsO_4 to *Periplaneta* was in direct proportion to the inhibition of reduced glutathione (GSH), and that they could be detoxified by injecting GSH. Anderson & Patton (2), however, discovered that $NaAsO_2$ in *Tenebrio* was not detoxified by GSH but by 2,3-dimercaptopropanol (BAL).

Forgash (80, 81) has investigated the greater arsenic-tolerance of female *Periplaneta*, which can withstand a 25 per cent greater dose of injected arsenious oxide than males, despite having no higher average concentration of glutathione in the tissues. He found that the arsenic combined with the GSH in the fat body more than in other tissues, and that this protective organ was eight times as massive in females as in males. The GSH was particularly concentrated in the mesenteron, which was 2.6 times heavier in the female than in the male and allowed 40 per cent less arsenic to be absorbed through it.

Thompson & Johnston (202) have reported that the developing eggs of a resistant strain of the blue tick *B. decoloratus* contained slightly more than twice as much sulfhydryl radical as a susceptible strain.

NEGATIVELY CORRELATED CROSS-RESISTANCE

Ascher & Kocher (8) discovered that the DDT-resistant Basel K_1 strain of house flies was knocked down by oral doses of alkali bromides much more quickly than a normal strain. Ascher (3) found that bromoacetic acid and its alkyl and aryl esters tended to be more toxic to two resistant than to two normal strains, the most clear-cut differential being given by cetyl bromoacetate, which had quite good residual action. Ascher (4, 5) then reported that cetyl bromoacetate (CBA) was more toxic than normal to a Diazinon-, a DDT-, and a chlordane-resistant strain, as well as a strain which had been submitted to pressure from chloroacetic acid but had not become resistant to it (15). Later, Ascher (7) found that the bromomalonates did not show this property, and concluded that CBA was the only bromoacetate that could overcome the lipid barrier in the tarsi of the resistant K_1 strain; however, he reported (6) that lauryl chloroacetate, which entered as a fumigant, was more toxic to the K_1 than to a normal strain. Bettini & Boccacci (15) pointed out that chloroacetate inhibited triosephosphate dehydrogenase, and Bettini, Boccacci & Natalizi (16) found that bromoacetates were faster insecticides than chloroacetates, just as they combined faster with SH groups. Bettini, Boccacci & Natalizi (17) confirmed that CBA was more toxic than normal to four resistant strains after two hours' continuous exposure, but when a 24-hr. observation period followed a 30-min. exposure they found that CBA was less toxic than normal to three out of the four strains.

Ogita (160) has discovered in *Drosophila melanogaster* Meigen that the dominant gene located at II-65 which confers resistance to DDT and other insecticides also confers resistance to phenylurea but unusual susceptibility to phenylthiourea (PTU); the dominant gene at III-50 which confers resistance to nicotine sulfate also confers resistance to PTU as well as to phenylurea. Ogita (161) found a combination of DDT and PTU that would kill every one of the four possible genotypes consequent on allelism in these two genes. Subsequently he reported (162) that PTU selection pressure restored the susceptibility to DDT and other insecticides, and subsequent pressure from phenylurea restored the resistance to them; pressure from both compounds produced nicotine-resistant flies. Unfortunately thiourea, a practical fly larvicide, did not show this negative correlation with DDT.

Mitlin, Babers & Barthel (148) reported that a technical grade of diisopropyl tetrachloroethylphosphate was several times more toxic to the Orlando resistant strain than to the standard NAIDM (National Association of Insecticide and Disinfectant Manufacturers) strain of house flies. Kearns (108) submitted a heterogeneous DDT-resistant population to selection pressure from this compound and found that it reverted to a susceptible strain in three generations. Attempts to reproduce a similar material with these biological properties, made at Beltsville, Orlando, and Riverside, have not been successful. Winteringham & Harrison (214) found that their diel-drin-resistant strain was much slower than normal house flies in recovering from anesthetics such as cyclopropane and CO₂.

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SOME ASPECTS OF THE APPLICATION OF INSECTICIDES^{1,2}

By R. J. COURSHÉE

*National Institute of Agricultural Engineering, Wrest Park, Silsoe,
Bedfordshire, England*

When entomological data have shown when and where to attack an insect pest and it has been decided that an insecticide is to be used, there remains the task of applying this chemical to the insect. So some of the steps between the stage when the insecticide is in the can and the stage when it is in the insect are considered in this review.

Application of pesticides is a subject concerned with several disciplines. However, it is generally accepted that many of the physical and engineering aspects can be considered in isolation from entomology and farming, providing they are dealt with in general terms. This separation is made in this general review, although the details of a particular example of insect control by chemicals would need to be viewed from a more complete basis.

Conventional methods of applying insecticides in agriculture appear to vary widely. Nevertheless, it is common to find that equally successful pest control is achieved when the chemical is applied with a bellows duster, an aeroplane, or in a number of seemingly different ways. Occasionally a known insecticide is found to be ineffective in the field. Then faulty application might be to blame. The following discussion of application is made mainly with those occasions in mind. Also, effective application methods may yet be relatively inefficient. That is, an equally successful biological effect might be achieved with a more efficient application method in which smaller amounts of insecticide are expended. Lower costs of pest control and a decrease in any hazard resulting from insecticide residues could thereby result, so that application deserves attention even when insect control is adequate.

AVAILABILITY OF PESTICIDE TO INSECT

It is useful to assume that an insecticide has a definable toxicity. A requisite quantity of available insecticide to cause the death of an insect may then also be defined. The quantity is not constant but may be considered approximately so.³

¹ The survey of the literature pertaining to this review was concluded in March, 1959.

² The following abbreviations are used in this review: BHC for 1,2,3,4,5,6-hexachlorocyclohexane, consisting of several isomers and containing a specified percentage of gamma; DDT for 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; DUE for deposit per unit emission.

³ Insecticide toxicities are often of the order of a few micrograms per gram of insect. A lethal dose for an insect weighing a few milligrams is then of the order of 10^{-11} kgm. To obtain this quantity on each insect roughly 100,000,000,000 times as much is applied to a hectare.

In the field it is only necessary to make the insecticide available to this sufficient extent for control to be achieved. If the insect is not controlled, then we can assume that the insecticide is not available. It may be in the wrong place, or in the right place at the wrong time, or in the wrong form. At any rate, it is somehow unavailable. This, then, is the subject of application: making pesticides available in a manner which increases crop yields through pest control. This must be done in an economically acceptable way.

Some indication of the availability of an insecticide might be obtained by measuring, for example, spray cover, spray-deposit density, or its distribution through the target area. These measurements have often been made to obtain some index of the performance of an application machine. The biological effect of the insecticide is, however, dependent upon the distribution of the insecticide on or in the insects, and this is very rarely measured. It is simply inferred that the insecticide is available whenever the insects die and vice versa.

COMPARISONS OF APPLICATION METHODS

In a review of application, it would be valuable to be able to quote the results of comparisons between different application methods. These comparisons should show how effective biologically the methods are and also provide information on the physical performance of the application machines. The cause of any biological difference might then be identified with physical differences. Physical differences might be common to a number of machines. If an important effect is found to be general, we can establish a principle of application which may then be utilized in another situation.

An example will help to clarify this. If, say, it is found that a fine spray is ineffective in controlling a certain pest under particular conditions, several reasons for the failure could be suggested. But if simultaneous measurements are made of how that spray fails to become available, it might be found that evaporation of the carrier liquid is to blame. Then evaporation of equally fine spray of other carrier liquids of similar volatility might be expected under similar conditions. What machine or what insecticide is used might not then be relevant. The underlying physics are of wider value if valid generalizations can be made.

There are dangers (including naive rationalism) in attempting to generalize in this way from specific examples, for exceptions can sometimes be found. In this example a deliquescent insecticide might upset any simple theory, but generalizations which are nearly always true are still helpful. So an attempt has been made to generalize from the data which is available. The alternative would be to report factual examples, but not make any attempt to speculate about the principles that they might demonstrate. Such factual reporting of any subject is often preferred for the reason that an ounce of fact is worth a ton of theory.

However, pure reporting is not yet possible in the subject of insecticide

application for two reasons. The first is an extreme lack of data. Although there are many biological comparisons between two sorts of machine, there are extremely few which provide also information on the insecticide recovery and its distribution on the pest or the host. The second is the difficulty of obtaining generally valid field data in the subject of application. For example, suppose it is desired to consider the suitability of a certain machine for the control of an insect. It might be possible to quote one particular relationship between the distribution achieved by it on a certain occasion and the control obtained. But this would be a true relationship only for the particular circumstances. Machine setting and the way the machine was used, weather and crop conditions, and still other factors would all need to be specified before the data could be significant in a general way. These two reasons—firstly that there is little data at all and secondly that even where it exists it is not often sufficiently detailed to be interpreted—prevent an empirical approach to the subject of insecticide application at present.

On the other hand, if application is considered separately from the biological effect of the insecticide applied, there is some evidence. For instance, there is information on the range to which an air stream can penetrate the wind to reach a tall crop. No matter which insecticide is being applied against what insect, this information on application alone is of general use when it is necessary to propel a chemical to the top of a tall target.

Much of the quantitative part of the following discussion is accordingly restricted to the bare physical and mechanical aspects of application. But these are subordinate to the biological specification of how, when, where, and in what form the insecticide should be applied. Any prediction from physical principles needs to be demonstrated biologically and therefore biological matters must also be considered. But in the present stage of development of the subject, this part can be only qualitative and largely hypothetical.

There are a large number of ways in which insecticides are applied. The three main ways, though, are run-off spraying, small-volume spraying, and dusting. Only these are considered in this review. Because the two spraying terms are used to denote a variety of spraying methods in various countries, they are redefined for clarity here in a way which is slowly finding acceptance in England (9).

Run-off spraying is the ideal form of large-volume or high-volume spraying. A sufficiently large volume of dilute spray is applied to saturate all the crop surfaces. A certain amount of spray, called the maximum initial retention, is retained and surplus quantities drain off. Accordingly, all the crop surface is contaminated with insecticide. Often the deposit density of insecticide obtained is proportional to the concentration of insecticide in the original spray but independent of the volume of spray applied. The ideal of spray running off all the crop surfaces is rarely obtained in practice.

The volumes of spray required are large, and they need to be directed thoroughly to all parts of the spray target. In actuality, large-volume spraying falls short of run-off spraying.

Small-volume spraying is the alternative. No attempt is made in this application method to cover all the crop surfaces. Instead a patchy distribution of insecticide is formed. Reliance is then placed on either movement of the insect to contaminated patches or redistribution of the insecticide so that it becomes available to the insect. In this method, deposit density of insecticide is usually proportional both to its concentration in the spray and the volume of spray applied. This one would expect, providing the other factors affecting application are kept constant. Figure 1 illustrates deposits of large- and small-volume sprays.

These two spraying methods and dusting are different from each other in several ways, so that they are not always equally suitable. Selection of the best method of applying an insecticide and of a best machine is the reason for studying application. For example, we might assume that a primary requirement in application is to get insecticide onto the crop. Since the chemical is expensive, this should be done with as little waste as possible. On low-growing crops, least waste often occurs with small-volume sprays.

Dust fails partially to impact or adhere. It is commonplace to find that only 20 per cent of the quantity of dust applied remains on the target crop. (Although this is a typical figure, recovery levels in a particular case depend upon the dust, the target, and circumstances.)

With run-off spraying part of the spray pours onto the ground. A wide range of figures are quoted from experimental results obtained with large volume sprays. But when fruit trees were sprayed, for example, with 200 gallons of spray to the acre, the volume found on the ground beneath the trees was 160 gallons when they were dormant but only 20 when they were in full leaf (11).

This loss to the ground is not caused only by run-off but also by spray passing between the branches. If 300 gallons were applied to smaller or larger trees, the losses to the ground might assume different values. Particular figures are valid only for the conditions of the experiment. Nevertheless, in large-volume spraying some loss of insecticide to the ground occurs from that part of the crop where the maximum initial retention is exceeded.

In consequence, the fraction of the pesticide landing on the target crop is often greatest when small-volume sprays are applied. Then loss from neither of these causes—failure to adhere or running off the crop—occurs. The drop sizes in the spray are usually in the range 100 to 300 μ diameter. This drop size range would probably cause only small alternative losses through drift or spray bouncing. Therefore, if deposition of the spray on the crop is to be taken as a main index of biological effectiveness, small-volume spraying is usually the most meritorious. However, the merit of an application method is not determined solely by its effectiveness. Further, effectiveness is not determined by deposition only.



FIG. 1. Large-volume spray deposit (grey blotches) together with a small-volume deposit (dark circles). Application rates were 200 gallons an acre and 6 gallons an acre.

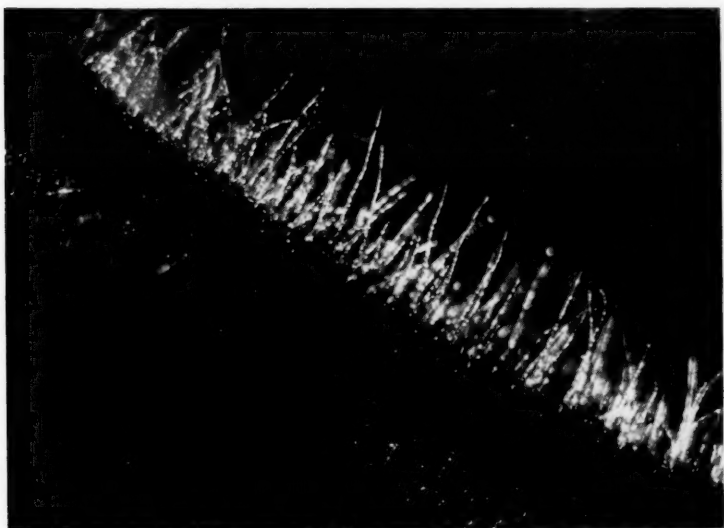


FIG. 2. Small drops of a malathion spray trapped on plant hairs.
(Photo, L. N. Staniland)

SELECTING APPLICATION METHODS

It was stated at the very beginning that equal effectiveness is often obtained with widely different applications. This is not always true, but even when it is, equal effectiveness does not always imply equal suitability. In either case there may be differences in the suitability of different application methods and machines. Then there must be ways of finding out which are best for particular purposes and circumstances.

A number of criteria might be used in defining the best method. Speed may be of high importance in one example, simplified equipment in another, or a degree of selective action in a third. These are, however, only partial steps aimed toward a final criterion which is, usually, over-all economic suitability from the farmer's viewpoint.

So if information can be obtained on the factors affecting profitability, a decision can be made. It is not always easy to obtain this information, particularly when several factors are involved. Still, it might be possible to decide which is better by a profit criterion: aeroplane spraying of a pesticide as an oil solution or spraying with a ground machine to run-off with the same pesticide, but probably in different quantity and formulated as a wettable powder.

The search for a best way of applying pesticides and for a best machine is perhaps more prominent in pest control than it is in other engineering studies, because biological effectiveness is only one of the factors which matter. A decrease in effectiveness might be accepted in return for some alternative improvement. More usually in engineering, a specification can be prepared for the performance of a machine. Then, selection is a matter of choosing the cheapest machine which provides the performance. In application of insecticides, performance is still a vague term and biological effectiveness is often a flexible index of a machine's performance. For example, a high rate of work might be demanded to cover a large area. In return, a less complete control over pests at any place in the area might be acceptable. Usually it is the whole picture which matters.

Two methods of selecting application methods are used. The more direct and widely used is the pragmatic one. In this, to take the previous example, if an aeroplane and a ground sprayer are compared in the field and the latter is less suitable on economic grounds, then that is the end of the study.

The alternative approach, an analytical one, is to try to find out why one is better than the other. To illustrate, if method A is better than method B, is it because A gives a better insect control? If it is, is this in turn because method A gives a higher recovery of pesticide, or is the chemical more suitably distributed over the target? Or is method A quicker and therefore more timely and cheaper on labour costs?

The pragmatic approach is of immediate practical value but only for the circumstances of the measurement. Unless many similar measurements are made under a variety of conditions it is difficult to foresee what further

change might be caused by altered circumstances. Frequently, the replication necessary for such measurements is too expensive to carry out.

The analytical approach also has shortcomings. For example, electrical charging of dust particles causes them to deposit more completely onto a target crop. This is a demonstrable fact, but it does not show that a farmer is better off with an electrostatic duster than he is with an ordinary one. Supposing it is desired to compare electrostatic dusting and small-volume spraying, which are rivals at present. The deposit of insecticide obtained from each method with equal expenditure would differ. But insecticide distribution and retention and also the control over most insects might also be dissimilar. Hazardous drift, working rates, and maintenance costs might vary with the two machines. Hence, a final decision on their relative merits which takes account of all the points of difference between them could not be reached easily. To suggest, as is sometimes done, that a generally valid decision can be obtained by a "practical comparison in the field" is sophistry.

It is this analytical type of study which is considered here. Although it receives and perhaps deserves the epithet "academic," it may be a working method superior to empirical testing, even in this practical subject of application. Ways of rendering insecticides available to insects are discussed, along with ways of selecting best methods, by weighing and balancing the advantages and disadvantages of alternatives.

This explanation of the reasons for the ways in which application is considered in this paper is necessary for the following reasons. Application is partly a physical subject and partly, at the beginning and at the end of an investigation, biological. Because it is wise to consider only the physical part in the middle in a theoretical manner, so attention is restricted to this area. Any theoretical interpretations drawn may then be acceptable. So the first reason is to forestall the pragmatist's criticism that theory does not work in entomology.

The second reason is that here application is made to look oversimple. In practice, the subject is complex because it is affected by many uncontrollable factors. Sometimes particular biological, meteorological, agroeconomic, and other factors play a part in individual cases of plant protection, and they cannot all be considered here. An attempt has been made to simplify the subject here by considering some major factors only in principle. This may help to explain application, but the difficulty of making use of the principles in the field should not be underrated.

ANALYSIS OF APPLICATION

As an example of the analysis of an application problem into its component parts, the spraying of sugar cane against various stem borers is considered. Kalra, Sidu & Harbans Singh (23) showed that a timely large volume spray of 0.02 per cent endrin applied as uniformly as possible on small plots as an emulsion with a hand lance gave a measure of control of several of the sugar cane borers and an increase in yield of the crop. (No measurements

were made of the retention of the dilute emulsion on the cane or of the location and magnitude of deposits of insecticide on the crop surface.)

An attempt was made recently to obtain a similar result with unskilled labour on a large scale in the field. No difference was then found. It was thought, but not shown, that both treated and untreated cane were as badly attacked as they probably would have been had no spraying been done. The plots were probably large enough to avoid interplot effects. Therefore, if the borers in the field are presumed to have been susceptible, like the borers on the research plots, to available endrin, it can only be concluded that in the field the endrin did not reach them.

In one instance, a field operator applied 0.02 per cent endrin at the rate of five Imperial gallons an acre. These contained 0.01 pounds per acre of active ingredient instead of the 0.2 pounds per acre contained in the 100 gallons more usually applied to obtain a large-volume spray deposit. His instructions were to prepare and spray 0.02 per cent endrin emulsion; this he did, and he cannot really be blamed. Evidently the command was not sufficient, and yet how often in advertising literature (even research papers) is the spray concentration quoted as a definition of the application. Some of the remaining work was done just prior to heavy rainfall. This may have affected the result, but no knowledge is available of the retention on cane of the particular formulation of endrin after rain.

The timing was late for most of the spraying and it may be that the hatched grubs had entered the cane before the insecticide was applied to the crop surfaces. A rapid means of spraying the area to be treated might therefore have been more valuable than the traditionally accepted hand methods which were mainly used. Aeroplanes were, in fact, called in and did spray, but the original specification was for a cover spray, not a small-volume spray which was all that the aeroplanes could provide. Also, at a shade temperature of 25°C. within half an hour of sunrise it is possible that only a small fraction of the emitted pesticide reached the crop because of evaporation of the carrier liquids and the consequent low impaction efficiency of the fine residual particles. Moreover, we do not know what the spray drop sizes were before or after evaporation.

The difference between emitting insecticide from the machine and applying it to the insect will be accepted by all, but it is as well to draw attention to the important distinction between emitting insecticide and applying it to the crop. The losses may be well known. But perhaps it is pardonable to mention them if it is emphasized that the whole subject of application is being considered here.

Finally, wherever as far as was known a timely application of endrin had been made in the absence of rain and according to the more complete version of the instructions, i.e., 100 gallons per acre of 0.02 per cent endrin, there was still, for unknown reasons, no control over the pest. Here we have an example of success in a treatment which was attained by a skilled staff

under favourable conditions, but less certain under the more exacting and less stable conditions in the field.

By now many of the defects leading to this failure have undoubtedly been eliminated by later experimenters. Moreover, this is an extreme example and it is much more usual, as suggested in the introduction, for widely differing application methods to have similarly successful results. But it suggests that application of insecticides should be considered to be more than a matter of logistics, that is, of supplying spray chemical and an adequate number of working machines and men. This is the obviously important task in the field after suitable applications have been designed previously on the research farm and tested in field scale trials.

This example illustrates vividly how little is sometimes known about applying an insecticide. An efficient application method for an insecticide might be defined as one which provides complete biological effectiveness, but a very efficient application method may not be an economic proposition, as the following example suggests.

Young and his colleagues (44) showed that, when applied to potatoes as a fine spray in 16 American gallons of water per acre with a specially made sprayer fitted with trailing drop legs, endrin provided a control better than 95 per cent over green peach aphid [*Myzus persicae* (Sulzer)]. The same amount of insecticide applied in a smaller volume of water from the air (that is, from above the plants as a coarser spray, giving a smaller recovery and less cover and also light underleaf deposits low in the plants) failed to control the insect as well. We have on the one hand an effective but slow and relatively expensive application method, and, on the other, a less expensive but less effective application by an air spray contractor.

Since the aphid is a virus transmitter, high effectiveness of the treatment is important. But it is not so important as to outweigh completely all other considerations. The value of nearly complete control needs to be balanced against the advantages of aeroplane spraying, particularly of this dense crop which is easily damaged by ground machinery. The aeroplanes provide speed and convenience and avoid mechanical damage to the crop. But they may require an oil-based formulation, be restricted by weather, and are expensive (in most countries). In the example at hand, they are also less effective, possibly because of decreased recovery of insecticide and inadequate distribution of it through the crop. The ground machines using a water-based formulation differ in each of these aspects. If no single feature is of overwhelming importance, a choice between the application methods needs to be made on the basis of a balanced view of their relative merits in the aspects in which they differ to an important extent.

Lest this approach sounds pedantic, let it be suggested that the most valuable first step to take is to make a list of points of difference which are of so little importance that they can be disregarded, leaving just the few which are essential. For example, aeroplane spraying usually leads to more

drift than spraying from the ground. If this drift causes no hazard downwind, then it is important only as a loss of wasted chemicals. It is possible, however, that drift can be reduced to 1 per cent of the application and a loss of pesticide of this magnitude is probably insignificant relative to other causes of its inefficient utilization. Drift, then, is usually the one item which may be cut off the list for examination. But, as the whole subject of downwind contamination arising from drift is scantily understood, it cannot be dismissed in an equally lighthearted manner if the pesticides cause harm.

The ground sprayer and the aeroplane will differ in many highly significant ways. Some of the probable variations which cause the discrepancy in biological effectiveness are considered next.

Recovery.—The recovery of a pesticide is the amount which can be located on the target after spraying and which is usually expressed as a percentage of the amount of pesticide emitted from the machine. It is determined by the deposition of the spray, by its distribution between target zones and other areas, and by its retention in those target zones where it might become available to the pest.

In work on spraying machinery, the elementary concept is generally used in the early stages. This is: if the target for the spray is believed to be a plant, then we measure how much of the emitted spray is found on the plant and how much goes elsewhere. Usually, increased recovery is an index of merit since less of the chemical emitted is then presumed to be wasted.

This simple notion of recovery of spray arose mainly with aeroplane spraying from a height of several metres. In this type of spraying the small drops which settle slowly may be blown out of the field being sprayed by the wind. So it seems worthwhile to measure the fraction of the spray which lands in the proper area. The spray is usually required to land on the crop rather than the ground between plants, so it is the recovery on the crop in the treated field which is important.

Typical measurements are given by Yeo, Akesson & Coutts (43). With an emission of 100 cc. of spray per yard of flight, peak deposit densities under the aeroplane were 3 cc. per square yard. The deposit was still as high as 0.1 cc. per square yard at points 100 yards downwind. These long-range deposits might be useful, but would be wasted if a small area were being treated. Some 45 per cent of the applied material was found on the ground between 10 and 1000 yards downwind in this example.

Courshee (11) considered the waste of insecticide in an analogous fashion on deciduous trees. Before the trees came into leaf, 70 per cent of fine spray (with surface-volume mean diameter 65μ) was wasted by drifting out of the half acre plot being sprayed by a mist blower. A coarse spray gave nearly no drift out of the plot at the same stage of development. None was detected, in fact. On the other hand, 70 per cent of the spray was found on the ground underneath the trees when a coarse spray (surface-volume mean diameter 250μ) was applied. Both the fine and the coarse spray were wasteful, but in different ways. These particular figures apply only in the

experiments just described. Quite different figures might well be found on other occasions. It is sufficient to say that of the spray in the tank, only a part reaches even the primary target.

However, there is really little interest in spraying crops. It is insects that the insecticide has to reach. The recovery of insecticide on sprayed insects has been noted only occasionally, mainly by those concerned with locust control (15).

A view of insecticide recovery which approaches this aim more closely than the elementary one of spraying the whole crop is used by Morgan (26). He showed that of all the BHC recovered on a sprayed apple tree, only the small fraction of it which seeps between the clusters of bud stems has any chance of affecting an oat apple aphid (*Rhopalosiphum prunifoliae* Fitch) hidden there. Therefore, recovery is valuable only in these small and particular areas.

Recovery of insecticide is presumed to be of importance in application since it must indicate the dose which an insect receives. After spraying, the insecticide which was in the tank should be found where it has a chance of becoming available to the insect. It should not drift into the next field and usually it is required on the plant rather than on the ground. If more complete biological information were available, it might be possible to pinpoint particular areas of the crop which are important targets. Unless the insecticide is recovered where it can be useful, its emission from the sprayer tank is merely a waste. Moreover the chemical should be on the target when it is useful and in a form in which it can persist and become available.

Incidentally, this is a convenient stage at which to re-emphasize that application must rely upon biological information about the times at which the pests are most easily reached with insecticides or are most susceptible to them. The best application method is not determined by this data alone. Mechanical and operational requirements also matter. Nevertheless, the biochemical information provides the basis for the application. Such questions as where should the insecticide be placed; when and for how long should it remain; should it be in solution or suspension; is a complete cover necessary, all help to decide how to view the importance of recovery of insecticide. This, in turn, is an index of whether or not a particular insecticide in a given form will reach each pest. It is this recovery on the insect which is important. Recovery of insecticide anywhere else may be only a step in the right direction.

So to reach an insect, the insecticide leaving the spray tank must land on the crop, be retained there, and be distributed through the crop wherever the insect is found. It should be in a form in which it can be readily taken up by the insect from wherever it is deposited. This approach is justified by the assumption, which seems reasonable, that enough insecticide must reach the insect to kill it.

The chances of sufficient insecticide reaching the insect are increased if the deposit density on particular targets is increased. This deposit on im-

portant targets should be attained with as small a consumption of insecticide as possible. Therefore a relevant measure of efficiency of application may be the deposit obtained for unit emission. From the initials of "deposit per unit emission" the term "DUE" has been coined. It might be found useful in experiments on pest control to measure the DUE.⁴

Landing on the crop or on the insect directly.—If spray drops or dust particles are moving in a stream toward a target, which might be a crop or an insect, then we should reckon that they would land on any object in their path. Usually they do, but sometimes they do not. Possibly the particles will land somewhere on the crop. If anywhere on the crop is a suitable target area, then deposition in this fashion is acceptable.

Supposing, though, that particular portions of the crop need to be sprayed. Then if the insecticide is prevented by some factor from reaching those parts, the pesticide is largely wasted even though it reaches other parts of the crop. Thus, in the example of aphids sheltering between the bud stems, a small-volume spray is inefficient as it is retained outside the cluster. BHC was used, and the vapour pressure of this insecticide might not be sufficient for fumes to reach the aphid from such a deposit.

Similarly, a number of factors affecting the landing of spray or dust onto the various surfaces of a crop might influence an insecticide's chances of reaching an insect. The most notorious of these is the effect of inertia forces on deposition efficiency. Deposition may be inefficient when small drops approach a target on a stream of air. The air divides to pass the obstacle and small drops may be carried round with it instead of proceeding on their original course and striking the object. Several sets of measurements have been made of this effect. A recent contribution is Jarman's (20), whose data is used to draw Figure 3. The tendency of the small drops to avoid landing on blunt targets usually allows them to penetrate with an air stream more deeply through a canopy of foliage (but Johnstone (21) has shown how this may not always be true). It may cause many of them to pass right through the canopy. Since they are small enough to drift, they may then be lost and, worse, cause a hazard downwind. Finally, small drops show a preference for landing on sharply curved surfaces. These parts of the plant may or may not be suitable receptacles for the spray. The trapping of fine spray on the stem hairs of a plant is shown in Figure 2, a photograph using a fluorescent dye which was taken by Staniland (38). A systemic insecticide might be unable to penetrate into the plant when perched on a hair.

If crop surfaces were similar to the artificial surfaces usually employed

⁴ The DUE and the recovery are closely related as follows:

$$\text{DUE} \times \text{area contaminated} = \frac{\text{recovery}}{100} \times \text{insecticide emitted.}$$

A DUE is easier to measure than recovery since no measurement is required of crop areas for the former. However, recovery provides more information to the machine designer.

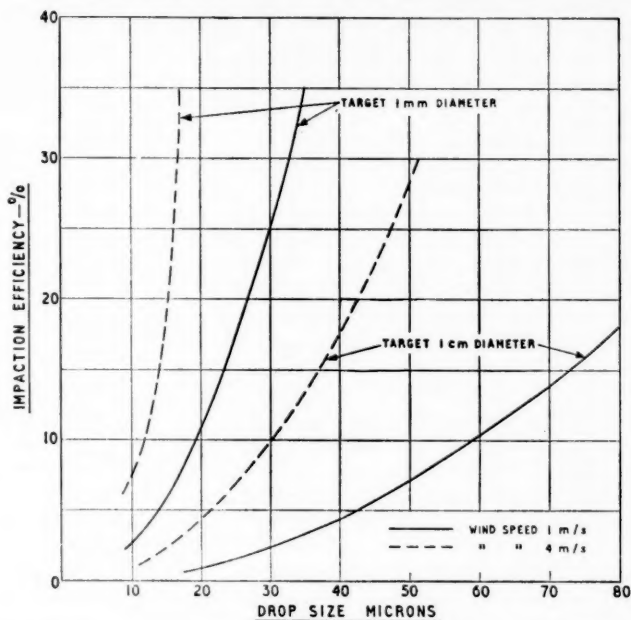


FIG. 3. The efficiency of impaction of small drops according to Jarman (19). Efficiency is defined as 100 times the ratio of drops which land over drops which would have landed if they had not been deflected from their original course.

in Jarman's tests, deposition of fine spray on them could be predicted from Figure 3. In fact, the surfaces resemble spheres, cylinders, and planes only approximately. In detail the surfaces are complex in form and nature, as Bradley & Juniper (6) have shown. Nevertheless, small drops do tend to land preferentially on sharply curved biological targets, whatever their ultimate surface texture. The photographs of deposition on hairs show this. Equally, they tend to avoid blunt surfaces and so penetration through a leaf canopy would usually be enhanced by use of small spray drops. One exception to this might occur when spray is projected without a supporting air stream, as small drops might then be diverted by wind (8). Coarse spray might also penetrate more deeply in a vertical direction if it is drifting toward a crop with many nearly vertical surfaces, grass for example. In this case fine spray might find more targets in its path than coarse spray would and less would penetrate to the ground (21).

Because very small dust particles a few microns in diameter may fail to land on any part of the crop, excessively large fractions of the insecticide

expended may fail to be useful. Splinter (37) suggests that in the field as little as 10 to 20 per cent of the dust emitted is often found on the crop. A specific figure like this is intended only as a rough guide. Nevertheless, it does suggest that incomplete deposition of particles smaller than $30\ \mu$ causes serious waste.

Hartley & Brunskill (17) have shown that large drops of high surface tension bounce from crop surfaces, particularly those with a bloom, e.g., cabbages. A typical result of their work is the following. Drops $250\ \mu$ in diameter falling at terminal velocity on fresh pea leaves are all deflected and are not retained if the spray surface tension is 57 dyn/cm. A lower surface tension or small drops permit the retention to rise—from 0 for $300\ \mu$ drops to 100 per cent for $100\ \mu$ drops. The latter is independent of the surface tension. In what is commonly called a fine spray, $100\ \mu$ to $300\ \mu$ drops are typical. Consequently, these drop sizes are widely used, and, on pea leaves at least, retention of them varies in the range 0 to 100 per cent.

As can be seen, bouncing might be an important factor in determining whether or not a drop of insecticide lands on other crops also. Inefficient impaction and bouncing from surfaces which are not readily wetted are general and they can have widespread effects. There seems to be also, though, a specific effect which controls the deposition of oil droplets on particular surfaces. Jarman (19) shows that typical artificial surfaces used to record spray, e.g., paper, glass, and soft layers of magnesium oxide, appear to receive between 6 and 100 per cent of the spray drops which approach them. These results were obtained for drops larger than $100\ \mu$ and might have been caused by drop bouncing. Although the various surfaces of a plant may appear to be similar to each other in their capacity to accept spray drops which approach them, it is not known whether or not they differ.

The question of deposition in general has been reviewed by Splinter (37). He was concerned with small drops which do not bounce and did not consider their capacity. He pointed out that whether or not a drop collides with a spray target depends only upon the forces which act upon it. Some act to promote the tendency to land and others hinder it.

The inertia of the drop approaching the target tends to carry it along its path despite opposing forces. Therefore as Jarman's results (20) show in Figure 3, high particle momentum helps deposition. The main interfering force is the sideways drag of the slip stream of any air moving past the obstacle. The greater divergence of the air as it moves around a larger obstacle provides a greater force to turn a given particle aside. Also, smaller particles are more readily turned aside by a given air movement than larger particles.

Finally, Splinter pointed out that electrical forces act on any particle which has an electrical charge. There are three of these forces. They are (a) the attraction between a particle and images of similar particles, (b) the force experienced by one particle as the result of the field generated by the cloud of particles, and (c) the force caused by the electric field created by

electrodes on the dusting or spraying machine. For example, Potter (31) measured the charges which occur naturally on spray particles. These are of the order of 100 e.s.u. per gm. of fine spray. They have no effect on the deposition of spray even in field strengths of the order of 100 v./cm.

However, electrostatic deposition is used industrially in cleaning air of particles and in spray painting. In this process, the charges on the particles are very much greater. Charging should be of value in depositing insecticides that will not deposit where they are required without extra help. Failure to impact occurs mainly with particles considerably smaller than $30\ \mu$ in diameter. These are usually dust particles. Nearly all commonly used sprays contain only drops large enough to impact with adequate efficiency on targets in their path, without requiring the help of an electrical charge. Hence charging is potentially valuable for sprays mainly if the electrical forces can be made sufficiently strong for drops to be drawn in toward a target which they would otherwise pass by. The drift and waste which might now result from spray's passing through the gaps between branches might be reduced by charging the particles to a sufficiently high potential. Underleaf deposits could also be made greater in certain circumstances.

It was shown in the experiments of Bowen, Hebblethwaite & Carleton (4) that when dust particles were charged the DUE was doubled by comparison with uncharged dust. This raised the recovery from the common level of 10 to 20 per cent up to 20 to 40 per cent. The electrical fields operate more intensely near sharply curved surfaces. Preferential deposition occurs, therefore, on leaf margins, stems, and hairs. There may also be a tendency for deposition to be heavier near the extremities of the crop canopy.

If all insecticide applications were made with large spray volumes and with a coarse spray which could be given a low surface tension, then deposition of spray would not be a problem. The spray leaving the machine would land on any target in its path. But because dusting, small-volume spraying, and sprays of higher surface tension are considered sometimes to be more valuable than large-volume spraying, deposition onto spray targets requires consideration.

Uniformity of spray distribution.—Run-off spraying leads to an equal density of spray on similar crop surfaces. Generally, this results also in an equal deposit density of insecticide. Fajans & Martin (13) have shown, however, that run-off may consist of a more dilute emulsion than that originally applied. So occasionally even this sort of spraying may not give uniform insecticide distribution. All practical forms of application also give rise to deposits of insecticide which vary widely from point to point through the target area.

It is reasonable to assume that variable deposits are wasteful. If the lightly dosed areas are adequately contaminated, insecticide is being wasted in the heavily dosed area. If the heavier deposit is required, the deposit elsewhere is inadequate. This is not an original thought, but it is still, after many years of discussion, only a hypothesis.

McKinlay's (25) results of spraying against *Earias* and *Heliothis* tend to support this viewpoint. When 300 gallons per acre were applied carefully with a hand lance to ensure thorough coverage, a more complete pest control was obtained than that given by small-volume spraying. Presumably the large-volume spraying gave rise to fewer underdosed bolls and therefore gave protection to a larger proportion of the crop.

In particular cases decreased uniformity caused by concentrating an insecticide onto some parts of the plant may be an advantage. The nymphs of *Empoasca lybica* (deBerg), for example, appear to feed mainly on the underside of shaded leaves on cotton at the high leaf temperatures found in the Sudan. It might seem best to apply most of the DDT, to which the nymphs are susceptible, to the areas in which they feed and to ignore the more exposed leaves.

Amsden & Courshee (1) showed, however, that the insects are also found on the upper surfaces of the leaves during the night. It is much easier to place an insecticide there efficiently. So providing that exposure to sunshine does not cause insecticide losses, an overleaf deposit high in the plant may be suitable. Spraying practice had in fact suggested previously that overleaf spray deposits of DDT would control the insect.

The merit of uniform insecticide distribution appears to need fresh consideration in each particular case. Valid generalizations are difficult to make, but one is that consideration of distribution emphasizes the rivalry between large- and small-volume spraying. The former tends to give a more uniform distribution of insecticide. But until the advantage presumed to result from the more uniform distribution is demonstrated, it is likely that small-volume spraying will be accepted because the resultant savings in time are easily appreciated.

The importance to be attached to the positioning of an insecticide in the plant depends mainly upon biological information on how to make sure that the chemical reaches the pest with a minimum of waste. Accordingly, there are two separate aspects of distribution. Any single pest moves over a limited zone. Therefore, for all pests to receive insecticide economically the quantity of insecticide within a zone should be enough and no more. The distribution from zone to zone is usually referred to as the macrodistribution.

It may be remarked, as an aside, that this is more clearly demonstrated by fungicides than insecticides. If a fungus spore is susceptible only to a fungicide which is closer than, for example, half a millimetre away, then there needs to be a sufficient deposit within each square millimetre. That is, the zone effectively occupied by the spore is 1 sq. mm.

There are also clear requirements for certain stationary insects. Norman (29) showed, for example, that a cotton whitefly nymph (*Bemisia gossypi-perda* Misra and Lamba) was killed only if DDT fell on, and not near, it. Therefore DDT should be placed on each few square millimetres of the parts of the plant which are likely to be infected. At the other extreme, Joyce (22) showed that jassids (*E. lybica*) affecting the same plant were

controlled with a volume application rate of as little as 1 l. per hectare. The computed spray cover corresponding to this was of the order of 1 per cent. This small volume was applied, in turn, with an aeroplane, with a boom sprayer, and with a drift sprayer. All three machines gave an equal biological effect—probably because the application was the same with all three, although the machines themselves varied.

The microdistribution of insecticide, on the other hand, is its distribution within the zone occupied by a pest. In the last example, the very sparse distribution over the many leaves on which a single jassid feeds seems to be unimportant. In general, it may not matter how the insecticide is distributed within a given zone, provided there is a sufficient amount of it. Exceptions would occur whenever the distribution within a zone affects, for example, the time taken for an insect to become contaminated. Microdistribution might also alter the persistence of an insecticide deposit, as the sixth figure of a paper by Rich (33) tends to suggest.

Uniformity of insecticide distribution between parts of the plant which are important targets and those which are not is closely related to the DUE. If the insecticide can reach an insect in only certain parts of a plant, then it is important that all such parts have sufficient deposits, and uniformity between the deposits is desirable. But by definition, any spray placed on the other parts of the plant is wasted. Therefore, both DUE and uniformity need to be high only on the particular targets.

Batchelder (2) studied the feeding and infestation zones of the European corn borer [*Pyrausta nubilalis* (Hübner)]. He showed that for an insecticide to become available it had to reach the moist zone of the leaf axil. An insecticide could be placed in each axil as it sometimes is by hand application methods even on a commercial scale. Certain insecticides, if they were deposited elsewhere, could act there, however, by some form of action at a distance. This is a convenient term to denote the ways in which an insecticide deposit sometimes exerts an effect beyond its apparent boundaries.

Redistribution which is not detected and measured may account for some of the signs of action at a distance. The remainder is possibly caused by insects' contacting the deposits and then withdrawing, so that the control only seems to be occurring elsewhere.

Systemic insecticides which are transported from the deposit sites to other parts of the plant illustrate redistribution in one extreme form. To some extent, these insecticides make the nature of the application machine unimportant. However, Hartley (16) has made the significant comment that when redistribution is controlled by the plant it may not operate usefully; the plant may place the systemic insecticide in parts where it is unable to do any good. Stem borers in particular seem to be little affected by most systemics.

Most insecticides appear to show some degree of action at a distance. Even Gusathion [Guthion; *O,O*-dimethyl-*S*-(benzaziminomethyl) dithiophosphate] which according to Unterstenhöfer (42), has a vapour pressure too

low to measure at room temperature, shows a slight degree of penetration from one side of a leaf to another. More volatile pesticides and those which have an appreciable solubility, either in water films on the leaf or in the leaf tissues, are presumably transported readily. Their movement to zones which seem to be uncontaminated might easily pass undetected.

Another fungicide example may at this point be pertinent to the subject of redistribution of insecticides. Butt (7) showed that captan, in the formulation which he used, was redistributed in massive form by water on the leaf. Whole particles of the fungicide were moved. Captan is nearly insoluble in water but must be sufficiently soluble in cell solutions to take effect. Very slight solubilities and very small vapour pressures might need to be sought to account for action at a distance of insecticides, if this is not accounted for by insect movement.

It is apparent that the need for spray uniformity deserves some thought in each case. Furthermore, it is necessary to know not only that uniformity is valuable, but also how valuable it is. Applications which provide deposit uniformity are likely to be less practicable and more expensive than less meticulous ones, and we need to know in each case the value of the advantages of the former.

Large-volume spraying often leads to more uniform distribution through a leaf canopy than that obtained with small-volume spraying, provided the canopy is open to the approach of suitably aimed spray. On the other hand, if there are obscured target areas, the spray, as Fryer *et al.* (14) for example has shown, is usually more likely to reach them if drops with low impaction efficiency are used. These manage to avoid landing on the outermost layers of the canopy, where larger drops would be trapped. This result is more often true for drops moving with a slight wind. On many machines a forceful jet of air deflects the leaves so that they present only their edges to the spray. Then the penetration into the canopy may be as great with large drops as it is with small ones.

Finally, if after the canopy has been penetrated, the drops proceed only under their own momentum, as in the spray from hydraulic nozzles, the large ones may penetrate better than small ones (8). This effect may arise because small drops lose their momentum quickly and are also deflected more readily from their original course by any wind.

Penetration through a leaf canopy illustrates how varied a problem application can be. Large-volume spraying in one case, small drifting drops in a second, and large, high-speed drops in a third may best provide penetration into different types of canopy in various circumstances.

The question of getting spray to the higher parts of a tall target has been studied by several people. Inadequate spray deposits in the tops of trees are probably the reason behind the broad suggestion of von Oppenfeld *et al.* (30) that pest control is inadequate on trees more than 20 ft. tall.

Preliminary results showing how powerful an air stream is needed to reach against the wind have been reported by Courshee (9) and are shown

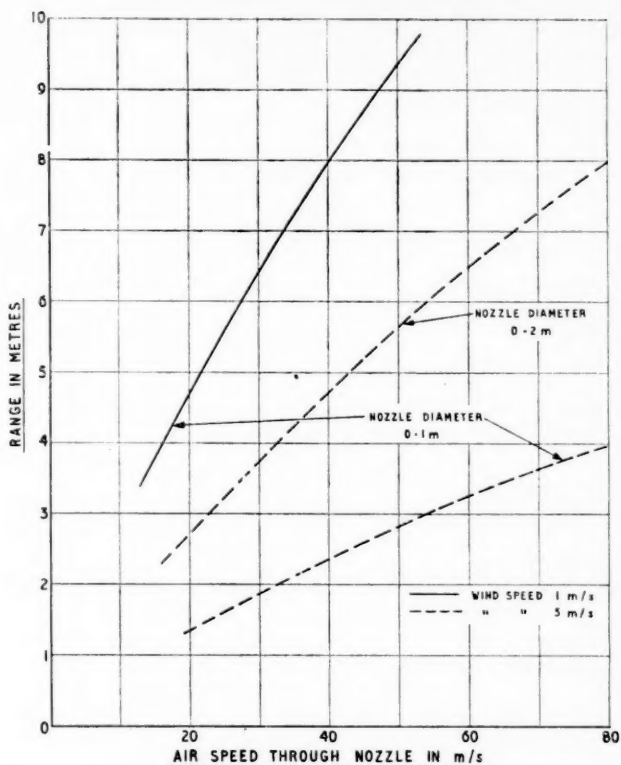


FIG. 4. The range attainable with mist blowers blowing against a wind. Practical range is taken as 0.7 times the ultimate range. (From original data obtained by Byass and Courshee.)

in Figure 4. There is less evidence for the range attainable across a wind and vertically. Brann (5) points out, however, that five to six times as much spray must be directed toward the top third of the trees as at the bottom third. Part of this requirement may be caused by the inadequate vertical range of many tree sprayers in the winds commonly encountered.

A last point on the question of uniformity of spray deposit becomes prominent whenever the insecticide spray has an effect on the crop independently of its action on insects. Usually such an incidental effect takes the form of damage from excessive doses, and overdosing is then at fault. Because overdosing normally arises only through unequal spray distribution, it can be a powerful argument for uniformity. Moore (27) suggests,

furthermore, that microdistribution of the spray may affect any damage caused to the plant by a given deposit density when lime sulphur is applied. Patchy microdistribution is said to cause damage with some insecticide formulations also.

Although a uniform spray deposit over the whole of the crop is usually valuable, occasionally it is not. Then a particular target on each plant needs to be contaminated uniformly for insecticides to be used economically. Since careful control over the uniformity of a deposit is often costly, it is necessary to compare its cost with its value. If it is decided that uniformity is needed, then the examples show that under differing circumstances different types of application method are likely to be most successful.

Retention, run-off, and persistence.—So far we have spoken of the deposition of spray. For small-volume spraying and most run-off spraying this is proportional to the deposition of the pesticide contained in it. It would seem reasonable to expect that, in general, pesticide deposit is equal to the product of volume of spray deposit times pesticide concentration in the spray. Rich (33) has pointed out, though, that for run-off spraying, the tacit assumption that pesticide deposit is proportional to spray deposit is not always justifiable.

Rich worked partly with Bordeaux. He showed that increasing concentration of Bordeaux did not give a corresponding increase of copper deposit, which tended toward a limiting value. This he attributed to saturation of the power of the negatively charged leaf to absorb the positive Bordeaux sol. Somers (36) was unable to reproduce Rich's result except when certain wetting agents were added to the spray. His results show that deposit formed by run-off is directly proportional to spray concentration.

One difference between their experiments is that Rich was doing large-volume spraying in the field and tacitly assumed, as Somers also did, that this was closely similar to run-off spraying. Somers, on the other hand, really was doing run-off spraying in the laboratory. It could, therefore, be that an alternative explanation of Rich's result is that at higher Bordeaux concentrations he achieved a smaller and smaller spray retention.

Somers' measurements and one of my own provide some indication of the exact nature of run-off spraying. In a check made with water, it was found that when run-off occurred from potato leaves held vertically, there was left a deposit of 20 mg. of spray per sq. cm. Somers obtained deposits of copper in the range of 10 to 20 μ g. per sq. cm., using a spray with a concentration of copper of 0.2 per cent by weight in water. Therefore the spray retained was in the range 5 to 10 mg. per sq. cm. providing the run-off was at the same concentration as the spray retained. Somers' result was obtained with vertical leaves.

Retention varies widely, but these figures of 5 to 20 mg. per sq. cm. give an idea of its order of magnitude. Ten mg. per sq. cm. is equivalent to 5000 l. of spray on a hectare, if the crop carries five hectares of leaf. To reach run-off

on the less accessible leaves and on the horizontal leaves and allowing for waste which is not a result of run-off, perhaps four times this volume would need to be applied, i.e., 20,000 litres per hectare.

Rich's plants might have been sprayed to run-off with only 250 gallons per acre (about one-tenth the volume given above), but it is possible that they were not and so the absorption theory may not be relevant. Somers' results suggest that the theory does not always hold good. However other workers, for example Hoskins & Wampler (18), obtained results similar to Rich's.

Clearly it is sometimes going to be valuable to measure both spray retention and pesticide retention in run-off spraying. On the other hand, it is not often that large-volume spraying rises to the level of run-off spraying in field applications. Therefore we can probably continue in the field to use the assumption that spray deposit is proportional to pesticide deposit. But both Rich's result (33) and Fajan's & Martin's (13) work should be borne in mind. The latter showed that oil emulsions sometimes deposit oil selectively, together with the insecticide dissolved in it, and the run-off consists of a more dilute emulsion. In this case oil deposits could build up even after the run-off stage has been reached.

Somers (36) made the comment that pesticide initial retention, for a given spray deposit, is dependent upon a number of ill-understood forces of attraction of the crop surface for the chemical. Ben Amotz & Hoskins (3), for example, showed how pesticide initial retention is altered in particular circumstances by altering the wetting power of the spray. They did not show whether or not changes in the spray initial retention were the cause of the altered pesticide initial retention.

Little is known also about long-term persistence of the pesticide. The term "retention" is reserved here for initial retention of spray, although it is often used to imply subsequent resistance of a pesticide deposit to weathering. Here this factor is called persistence so that these two aspects of application may be distinguished.

A number of mechanisms cause the loss or decline of activity of a pesticide deposit some time after it has been formed. Occasionally it is desirable to have a highly persistent deposit.

Rich (33) provided evidence of how complex persistence may be. He worked with fungicides, and one of them, Bordeaux, has a gelatinous structure; the other material was zineb. The loss of the latter in a given spell of weathering was proportional to the density of the deposit. A heavier deposit lost more zineb than a light deposit during the same period. This is the sort of result one might expect intuitively. But for Bordeaux the loss was not dependent upon the original deposit density. A heavy deposit lost no more copper than a light deposit in the range 1 to 10 μg . per sq. cm. These two and yet other different processes of weathering might be found also among insecticides. Loss of insecticide is often associated with rainfall, although many

insecticides are soluble to only a small extent in water. Therefore, unless they become soluble in a solvent formed on the leaf by rainfall, they can be lost through rain only by some process of erosion.

A very large amount of work has been concerned with sticking pesticides to crops. Resinous additives are described by Sullivan (40), for example. No general account of the value or action of such materials is available. Moreover, the additives also affect spray retention and deposit of pesticide. More data is needed to resolve a complex process. The application aspects, retention, and persistence of the very broad art of pesticide formulation are slowly being turned into a science, but there remains a great deal to do.

Once the insecticide is placed on the plant, it still has to reach the insect and get to the site of action within it. This part of pesticide application is not considered here since it lies beyond the stage which concerns spraying and dusting machines. (I feel, however, that the step in application from insect environment to site of action within the insect is one which is often critical and little understood.) Most of this essay is not directly about such machines but about finding out what they might have to do. The application machines have been considered briefly by Ripper (34) and by Brann (5). Spraying machines have also been studied extensively by Marshall (24) for the special case of fruit trees and more generally for a wider range of crops by Potts (32). The variety of spraying and dusting machines is very wide and considerable space would be needed to consider it. So machinery cannot and perhaps need not be discussed here except for the following comment.

It was suggested in the introduction that interest in application arises from the necessity of deciding what sort of application and machine to use. This decision must be based partly on consideration of the performance of a machine. Such consideration can be given only when standards of performance have been established for each biological requirement. But the choice also depends upon the many other factors which render a machine acceptable or unacceptable in given circumstances. These may differ significantly, even for single farms. So it is only rarely that a wise choice of machine can be made easily.

EXPERIMENTAL METHODS

A few experimental methods have been found to be of value in some of our work on application. The first is a philosophical point and the main tenet of this essay. It is perhaps stressing the obvious, but it does seem that a larger portion of the work intended to be on application should be concerned with application. More usually it is now concerned with pest control, which is a measure of insecticide availability closely connected with application. But if the control is inadequate, then measurements of pest numbers do not tell us whether or in what way the application has failed. The pest population can be a measure only of a successful application. That

is to say, failure to control a pest might result from many factors besides application.

It therefore seems preferable to work in this way: Once it is established that an insecticide formulation is active, decide how, when, and where to apply it. The decision depends mainly on entomological data. At this point the chemical can be applied in a variety of ways to see whether or not any particular mode of application is superior biologically. Such experimentation is often performed most easily on a small scale so that the application can be controlled accurately to measure the amount of insecticide per unit area and to give a precise degree of cover and a known distribution. The scale of working should perhaps be the smallest which does not materially distort the biological picture.

The next problem is to obtain the desirable application on a large scale. An insecticide is often unnecessary for this work, and some readily detected substitute chemical can sometimes accelerate progress. Field work on that application which is thought necessary for biological reasons might provide evidence in favour of other methods of application. Only now is a full-scale

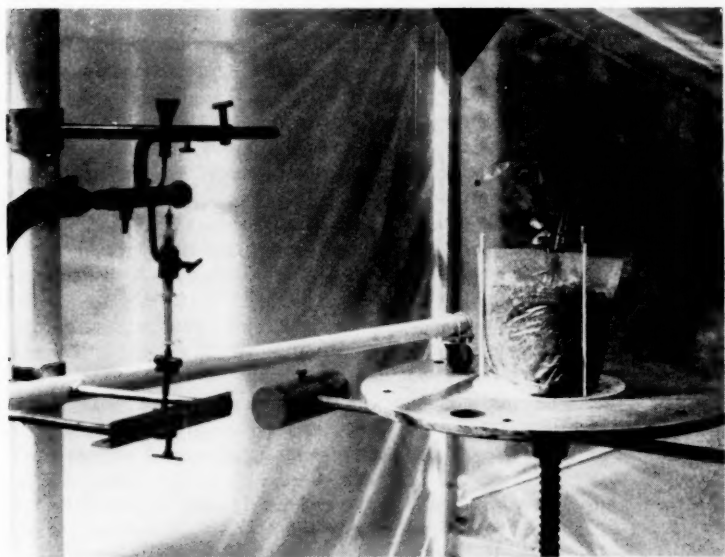


FIG. 5. A small sprayer in a wind tunnel. The plant is turned on a table which lowers gradually. Its movement causes a syringe to discharge into a miniature hypodermic needle nozzle. A controllable air blast allows selection of a required drop size in the range 50 to 500 μ .

check of the chosen insecticide, formulation, and application method needed. This is very costly work if it is done completely.

Information should also flow in both directions between the laboratory and the field. Unfortunately, information obtained in field-scale trials is rarely reliable enough to guide the more precise small-scale work. Consequently, precision laboratory sprayers and small wind tunnels are used widely; an example of our own is shown in Figure 5.

If the procedure just outlined is used, the following points are corollaries. First, field-scale application is often best considered in isolation from insects and insecticides in the initial stages of the work. This may be less true of large-volume spraying than of small, but if the main part of application is putting liquid drops or dust particles onto certain surfaces with machines, then it is as well to use a convenient tracer which can be easily measured on those surfaces. Calibration can indicate whether the replacement chemical behaves in the same ways as the proposed insecticide.

Second, if a tracer is used, quick, and sensitive measurement techniques are possible. In early application efforts, tracers were coloured dyes. These, and occasionally coloured insecticides, can be measured colorimetrically (15). Sharpe (35) used fluorescent tracers, and Staniland (39) has shown how such a tracer can be chosen to resemble an insecticide closely or even made to form a physical bond with it. Radioactive insecticides have been used widely in the laboratory by, for example, Thomas (41). Recently short-life isotopes have been employed by Nordby (28) to measure the spray distribution obtained with small mist blowers on apple trees in the field.

If a tracer is not used, chemical measurements of the insecticide are needed. Such measurements are relatively laborious and insensitive. They are also, perhaps, more accurate than they need be.

Third, the use of easily visible spray or dust deposits allows visual assessment of their distribution. This technique has been employed widely and developed by Davis & Elliott (12). Recently Courshee (10) has considered the accuracy attainable by visual assessment, and has also developed a technique for convenient periodic calibration of visual estimates of spray density. In this, visual assessment is used to make only relative measurements of spray cover, particle density, or insecticide deposit density. Absolute measurements are made only with instruments of known accuracy. Some of the objections to visual assessment are removed by Courshee's procedure.

Because spray deposits in the field vary widely from one leaf to the next, large samples are needed to measure the deposit and its distribution. Quick methods of measuring are essential. These should also be sensitive, as deposits are of the order of 1 μ g. pesticide per sq. cm. Both spray tracers and visual assessment permit more rapid measurement than many other methods.

CONCLUSION

Probably most applied sciences swing back and forth between alternate phases—between, on the one hand, hit and miss methods of investigation

with high initial success and, on the other hand, attempts to understand how a result occurs. Probably the application of pesticides has now again reached a point where the latter approach is likely to be more profitable for some time to come. This is especially true wherever small-volume sprays are applied. But run-off spraying still poses the several problems which were being considered at the time when it tended to become uneconomic and fell out of use. A consideration of both these application methods and of dusting suggests that the journey of an insecticide from the can to where it can reach an insect is occasionally not a straightforward one.

ACKNOWLEDGMENTS

Several people kindly criticized this paper. I am particularly indebted to W. M. Carleton, J. G. Horsfall, R. A. Galley, E. Holmes, H. Samuel, D. Yeo, G. S. Hartley, E. Pearson and his staff, D. W. Evernden, W. H. Vanstone, and also to L. N. Staniland for permission to use Figure 2.

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CITRUS INSECTS AND MITES^{1,2}

By L. R. JEPSON AND G. E. CARMAN

University of California Citrus Experiment Station, Riverside, California

As recently as 1950 and 1951, two comprehensive treatises, *Subtropical Entomology* by Ebeling (66) and *Citrus Entomology* by Bodenheimer (18), have fully discussed the status of various citrus pests throughout the world, the basic information concerning the biology, physiology, and ecology of each, and the methodology of citrus pest control. Concurrently Boyce (20) published an excellent and inclusive review of the entomology of citrus.

In view of these earlier publications it has appeared appropriate to limit this review to a consideration of the more significant contributions accrued during the past decade with respect to citrus pests, their biology and their control. Furthermore, it has not been considered expedient to discuss studies primarily concerned with biological control since this particular phase of citrus entomology has more recently been adequately reviewed by Clausen (38).

Even with these limitations as to subject field, it has not been feasible to discuss with adequacy all the contributions made during the review period and the following presentation will only provide some insight into the general trend of progress made in this area of study.

SCALE INSECTS

Scale insects are responsible for some of the most serious entomological problems encountered in the cultivation of citrus crops in the producing areas of the world. Their importance is assessable to the pronounced effect they may have on the productivity of the citrus tree (74) and the impact of their presence on the marketability of the fruit. Their world-wide significance can best be appreciated by an examination of the distribution maps for eight of the more important species included in the series prepared by the Commonwealth Institute of Entomology (39).

As evidence of the continuing importance of scale insects over 200 scientific articles, exclusive of those principally concerned with biological control aspects, have been published during the past 10 years reporting the results of studies on the biology and control of scale insects on citrus.

Unfortunately, only a few of these papers contribute significantly to a knowledge of the biology, ecology, morphology, and physiology of these species. The greater number are primarily concerned with control practices, a limited number reporting specifically on factors relating to hydrocyanic acid (HCN) fumigation or petroleum oil spraying, and by far the greatest

¹ The survey of the literature pertaining to this review was concluded in March, 1959.

² The following abbreviations are used in this chapter: DDT for 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; HCN for hydrocyanic acid

number summarizing studies completed with parathion and related organic phosphorus-type compounds.

This notable preoccupation with the evaluation of parathion and similar compounds is readily understandable even if not fully commendable. The dependence of citriculturists on HCN fumigation or petroleum oil spraying for scale control has become increasingly unsatisfactory. As early as 1916, Quayle (181) verified the resistance of California red scale, *Aonidiella aurantii* (Maskell), and black scale, *Saissetia oleae* (Bernard), to HCN fumigation. The eventual extension of the areas afflicted with these resistance problems and the later encountering of resistant populations of citricola scale, *Coccus pseudomagnoliarum* (Kuwana) (182), and of yellow scale, *Aonidiella citrina* (Coquillett) (151), greatly limited the usefulness of this control practice. Concurrently the costs of HCN fumigation increased so greatly as to challenge the economic feasibility of the practice. Added to these considerations were the risks of extensive, unpredictable tree damage and the costs of supplemental treatments to control the increasing complement of other serious citrus pests not affected by the fumigation treatment.

The effectiveness of petroleum oil sprays is not known to have diminished during the extended period of its use, but the deleterious effects of oil applications on the quality of fruit have been almost universally acknowledged. In addition, in some citrus growing areas, petroleum oil sprays too frequently contributed to heavy leaf drops, losses of immature fruit, twig die back conditions, and retardation of fruit maturity. The interest in improving the effectiveness of oil sprays, particularly by fortification with toxic additives, was transcended in many quarters by an interest in the availability of a nonoil spray treatment for scale control.

Thus it was not surprising that the disclosure by Schrader (198) of the insecticidal properties of parathion and the ensuing laboratory elucidations of its effectiveness on coccids set in motion a far-reaching evaluation of its suitability for use in controlling scale insects on citrus in various parts of the world. Field trials initiated as early as 1947 and rapidly expanded in the following years resulted in recommendations for field use in California in 1950 (32) and in Florida (82) in 1951. Concurrent studies in South Africa, Australia, and other citrus-producing areas similarly formed the basis for local recommendations to growers.

Parathion.—The overall usefulness of parathion as a control treatment for scale insects on citrus is recorded in the detailed studies reported by Cressman and co-workers on *A. aurantii* and *S. oleae* in California (44 to 47); by Carman, Ewart, and co-workers on California red scale, yellow scale, purple scale [*Lepidosaphes beckii* (Newman)], black scale, citricola scale, and cotton-cushion scale (*Icerya purchasi* Maskell) in California (32, 34, 36, 75), by Thompson, Long, and co-workers on purple scale and Florida red scale [*Chrysomphalus aonidum* (Linnaeus)] in Florida (154, 221, 222); by Naude and co-workers on California red scale, Florida red scale, and purple scale in South Africa (170, 171); by Johnston and by Hely on California red scale

and yellow scale in Australia (111, 139, 140); and by Costantino on dictyospermum scale [*Chrysomphalus dictyospermi* (Morgan)], black scale, and purple scale in Italy (42). Others working on various scale species in these or other citrus producing areas have made in many instances equally important contributions. Additionally, parathion has been reported as an effective control for several species of mealybugs which infest citrus (32, 115, 149, 153, 154, 164, 170, 199).

In general it was found that parathion was an effective scalicide used alone or in combination with petroleum oils. Mild phytotoxicity to citrus trees was encountered in some studies, usually involving only the loss of the older leaves (32, 47, 94). More prohibitive injury following the use of parathion and oil combinations on oranges has been reported by Carman *et al.* (34), and this combination is specifically not recommended for use on navel oranges (72).

Dosage requirements varied according to scale species, stage of development, and geographical area, considerations not meriting amplification in this discussion. In oil combinations the amount of parathion was increased as the amount of oil was decreased. Cressman (44, 47) has shown that even a special light grade of petroleum oil was suitable for use with parathion and Carman & Ewart (32) suggested the use of kerosene and parathion in combination. Most workers have concentrated on petroleum oils of heavier viscosities but frequently noted an accentuation of adverse tree reactions if such injury occurred following the use of the oil by itself. In this regard Cressman *et al.* (47) observed a relatively greater accentuation with light oil than with medium or heavy medium oils.

Combinations of parathion and petroleum oil were used in the same manner as oil sprays, both in timing of applications and in degree of spray coverage. On the other hand, applications of parathion could be made at any time of the year and with a modified coverage requirement. However, it was found by several investigators that applications immediately after bloom were most effective (32, 158, 171, 232). Against scale insects with even brood development, it was more advantageous to treat after the completion of hatch regardless of time of year (32, 75, 218). Moreover, Anderson & Atkins (2) noted the prohibitive toxicity of parathion to honey bees in groves and the advisability of avoiding applications during the bloom period. Results of the work by Cressman (43), which indicated that immediate postbloom applications of parathion resulted in a reduction of crop as compared to prebloom applications, are not conclusive.

Several workers (32, 84, 97) have demonstrated that a suitable distribution of parathion spray droplets is sufficient for adequate scale control. Griffiths *et al.* (97) in Florida found that parathion was equally effective for purple scale control when applied as a dilute or as a concentrate spray even through the amount of parathion per tree in the latter case was reduced by one-fourth. In California, Carman & Ewart (32) recommended the use of spray-blower equipment, applying approximately half the gallonage nor-

mally required for full coverage spraying in a single application or one-tenth the normal gallonage in each of two applications about a month apart. Since accelerations of dosage were not required in either case, the amount of parathion per tree was drastically reduced.

Insufficient evidence has been developed to indicate whether or not parathion functions partly as a fumigant after application to tree surfaces. The studies by Carman *et al.* (35) indicated that parathion did not occur as a vapor in sprayed groves in detectable amounts. However, Ewart *et al.* (75) attributed the kill of the smaller stages of citricola scale on untreated trees adjacent to treated areas to the drifting of spray material and the toxicity of parathion vapor. Furthermore, Hepburn & Bishop (115) observed that purple scale eggs, escaping the fumigant action of parathion, hatched, but the immature scale finally died, probably as a result of ingesting sublethal doses from the rind.

The slowness with which parathion manifests its ultimate effects on scale populations has been noted by several investigators. Equally apparent has been the differential kill on various tree surfaces. Data published by Cressman *et al.* (45) are representative, showing that parathion was most effective in killing California red scale on the gray wood, and least effective against scale on fruit surfaces, with the effect on scale infesting green wood being intermediate. When parathion was used in combination with oil, such differences were not observed.

Residues of parathion have been found on and in the peel of harvested citrus fruits but not in the pulp (11, 33, 75, 206, 209). Griffiths and co-workers (96) reported no off-flavor imparted to orange juice derived from parathion-treated fruit. On the other hand, numerous workers have found the fruit quality of parathion-sprayed fruit superior to that of oil-sprayed fruit [Bartholomew *et al.* (12); Harding (108); Sites (204)]. In California, Riehl & Carman (186) also measured a significantly lower incidence of water spot on parathion-sprayed navel oranges.

Parathion has been shown to be highly toxic to many parasites and predators, Bartlett's (13) controlled studies positioning it second after DDT in an array of commonly used toxicants. However, in field use on citrus, serious biological upsets have not been numerous. The adverse effect of parathion on the parasites of the soft scale, *Coccus hesperidum* Linnaeus, coupled with the fact that parathion does not appreciably inhibit the choline esterase of this scale insect [Metcalf & March (162)], has resulted in the significant increase of populations following parathion applications (15, 47, 68, 69, 75, 94).

Other organic insecticides.—The introduction of parathion prompted the objective search for a related compound with high insecticidal effectiveness but less toxicity to warm-blooded animals. Of the earlier compounds examined, malathion most nearly possessed these unique qualifications. In initial studies on citrus, Carman (30) found malathion to be a suitable substitute for parathion in treatments for the control of California red scale

although dosage and spray coverage requirements were higher. Similar effectiveness was reported for the control, in California, of yellow scale, purple scale, black scale, cottony-cushion scale, and citricola scale (31, 73). Johnson & Thompson (137) and Spencer & Selhime (207) reported favorable results in Florida in the control of purple and Florida red scales and purple scale, respectively. In interesting contrast to parathion, malathion was found by Elmer & Ewart (68) to be promising as a control for soft scale. However, mixtures of malathion and parathion were even more effective. This mixture and a combination of malathion and petroleum oil were also effective against California red scale (30), as well as yellow scale, citricola scale, cottony-cushion scale, purple scale, and black scale (31, 44, 73, 221). Serious population levels of cottony-cushion scale have developed in untreated grove areas subjected to malathion drift from treatment applications in adjacent areas (73). Bartlett (14) has shown that malathion is particularly toxic to the vedalia beetle, *Rodolia cardinalis* (Mulsant).

Results of tests with other organic phosphorus-type compounds have in some instances indicated promising results, but the studies are largely preliminary (40, 64, 207, 221, 226, 228). In other studies Fukuda *et al.* (87) reported favorable results with β -fluoroethyl fluoroacetate and monofluoroacetamide against the smaller stages of *Ceroplastes rubens* Maskell and *Unaspis yanonensis* (Kuwana). In Australia, Hely & Levitt (114) and others (67, 119, 156) have found sprays of sodium carbonate, alone or in combination with petroleum oil, effective for the control of the white wax scale, *Ceroplastes destructor* Newstead.

Petroleum oil sprays.—Investigations since 1950 on the use of petroleum oil sprays for scale control on citrus crops have been principally concerned with (a) the evaluation of organic phosphorus-compound additives to petroleum oil sprays, (b) the assay of narrow-cut fractions of naphthenic and paraffinic base oils, and (c) the more precise appraisal of the deleterious tree and fruit effects of petroleum oil applications. The use of parathion or malathion in combination with petroleum oil has been discussed above.

Riehl and co-workers (185, 187, 190) have published the results of various studies based on narrow-cut fractions of two stock petroleum oils representing distinctly naphthenic and paraffinic types. The carefully prepared fractions were treated exhaustively with sulfuric acid to free them sufficiently of unsaturated compounds, and the resultant oils fully characterized with regard to their physical properties before being used in laboratory and field tests.

The tests on California red scale clearly indicated the superiority of the paraffinic-type oils as well as the correlation of increasing effectiveness with increasing molecular weight within the range of 220 to 360 M, the correlation being better with the paraffinic-type oils than with those of naphthenic composition (187). Adverse tree effects, as measured by leaf drop on oranges and reductions of the juice quality of lemons and oranges, were not significantly different in comparisons with the narrow-cut fractions and conventional

oils. However, in later studies Riehl *et al.* (193) reported significant reductions in orange juice quality with the use of unfractionated, highly paraffinic-type oils at reduced dosages as compared with conventional oil spray treatments. Such reductions were not encountered in the use of an isoparaffinic oil of relatively low molecular weight. Differences in leaf drop were not appreciable between naphthenic and paraffinic oils although there was some tendency toward more leaf drop with increasing molecular weight in the range of 200 to 350 M. Differences affecting the quality of the juices were not observed.

The adverse effects of the currently available petroleum oil sprays on citrus trees and on citrus fruit quality have been studied extensively (191, 192, 201, 217, 224, 226, 227, 231, 236). Recent studies have stressed the means of minimizing these adverse effects, especially by proper timing of applications and use of certain plant modifiers.

Late summer and early fall applications on oranges and late summer to early winter applications on lemons have generally been found to be most satisfactory (189, 192, 193, 217, 227, 229). Stewart *et al.* (214) found the inclusion of 2,4-dichlorophenoxyacetic acid in petroleum oil sprays additionally helpful, particularly as a means of ameliorating certain adverse tree effects.

HCN fumigation.—During the period encompassed by this review, fumigation of citrus with hydrocyanic acid gas declined markedly as a control practice and relatively little investigational work has been reported. The interesting historical account of fumigation in California by Woglum (235) is distinguished no less by its informativeness than by its timeliness.

Singular attention should be called to the consummation of studies by Yust and co-workers (238, 239, 240) involving HCN-resistant strains of California red scale. These workers found that 40 "repeated" fumigations at 77° F. of second-moult scales of the originally resistant strain increased the resistance twofold and as much as fivefold at 59° F. with 43 fumigations. Resistant strains maintained in the laboratory through 49 generations without fumigation were only slightly less resistant (239). In other studies Yust (240) obtained evidence to suggest that resistance is associated with a high degree of dependence on metal-free, autoxidizable, respiratory enzymes, possibly of the flavoprotein type. No observable scale reaction accounting for resistance to HCN was found, although tracheal closure varied with different strains during fumigation (238).

Spraying techniques.—With spraying for scale control progressively supplanting fumigation, more attention has been given to the techniques of spraying citrus (29) and the feasibility of utilizing mechanical spray equipment. Lewis *et al.* (150) and Carman (29) have described mechanically oscillating boom sprayers predicated upon the development of high-capacity, high-pressure centrifugal pumps and the disclosure by Randell (183) of a unique method of nozzle oscillation.

Another type of spraying technique has been suggested by Hepburn & Bishop (115) as a means of integrating chemical and biological control. To avoid the build-up of soft scale in groves to be sprayed with parathion, they recommended spraying alternate sections of approximately 10 rows and completing the application on the initially untreated sections four to six weeks later. DeBach and co-workers (50) in studies on purple scale proposed a modification of this procedure using petroleum oil sprays.

Other scale investigations.—Some of the other papers reviewed emphasized, totally or in significant part, purely biological studies or were designed to support control studies. As an example of the latter, Spiller (208) studied the distribution of California red scale on leaves and found that the data were in agreement with the hypothesis of truncated lognormal distributions. The importance of such contributions, which may ultimately serve as a basis for standardization of population evaluations in scale studies, should not be minimized.

Dickson (54) in studies of the construction of the scale covering of California red scale noted that the covering was formed from cast skins (8 per cent by weight) and material secreted in the form of filaments by the glands of the pygidium. The secretions consisted of waxes (45 per cent) and proteinaceous material (47 per cent). The pure wax had a melting point of 86°–88° C.

Investigations suggesting why scale may not survive on presumably suitable hosts have been reported by Steyn (215) and Fukuda & Koremura (85, 86). Steyn concluded from his studies that California red scale development on lemon seedlings was retarded by a high nitrogen and low calcium content in the soil. Working with *Unaspis yanonensis*, Fukuda & Koremura (85, 86) found, through chromatographical analyses of leaves, that the factors responsible for the relative resistance of *Citrus natsudaoidai* as compared with the Satsuma orange were the low total nitrogen, amino nitrogen, and polypeptide nitrogen contents and the high organic acid content. The higher the scale populations on *C. natsudaoidai* leaves, the greater were the amounts of nitrogen compounds and potassium, and the smaller the amounts of phosphorus, calcium, and magnesium.

Of the several studies on the life cycle and seasonal development of scale insects in specific areas, the work of Pratt (179) in Florida on Florida red scale and purple scale is possibly of most general interest. From periodic scale surveys and weather reports received from various areas within the state over a period of several years, Pratt was able to correlate scale development with prevailing weather conditions and ultimately to predict the ascendancy and descendancy of scale populations. Florida red scale populations in the fall were correlated with the amount of cold weather experienced in the preceding January through March period and negatively with the rainfall during the same period. Purple scale populations in December correlated with the amount of warm weather the previous December and

January. Populations in May were proportional to those in December unless there had been a spring drought or late cold period (in which case the population would be higher) or unless there had been an abundance of spring rain (in which case the population would be lower).

Studies of presumably more local interest, involving seasonal influences on the life cycle and development of scale insects, include those of Minatta (163) in Argentina on California red scale; Gerhardt & Lindgren (92) in California and Cabido Garcia (25) in Portugal on dictyospermum scale; Monastero (165) in Italy on Glover scale, *Lepidosaphes gloverii* (Packard); Kozarzhevskaya (144) in Abkhazia on *Leucaspis japonica* Cockerell; Simmonds (200) in South Australia on black scale, Canceleda da Fonseca (27) in Portugal on soft scale; and Smirnoff (205) in Morocco on purple scale, Glover scale, dictyospermum scale, and black parlatoria scale, *Parlatoria zizyphus* (Lucas).

FRUIT FLIES

Some of the outstanding discoveries in economic entomology of the past decade have resulted from studies of lures for fruit flies, a number of which are major citrus pests. In regular control programs the incorporation of a lure in the spray makes it possible to control the insect with less than complete coverage applications, and effective lures in baits may be used to control fruit flies by treating only the nonfruiting areas of the host plant. Any insect eradication program is to a large extent dependent on survey and detection methods; therefore, all such programs can be made more efficient and less costly by the use of attractive practical lures. This has been very evident during the 1956 Mediterranean fruit fly, *Ceratitis capitata* (Wiedeman), campaign in Florida (104).

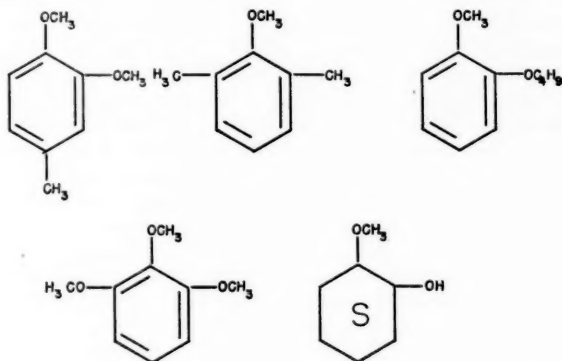
In basic nutritional studies of food requirements of the oriental fruit fly, *Dacus dorsalis* Hendel, Hagen (103) found that certain enzymatic yeast or soy hydrolysates contained elements essential to the normal development of this fruit fly and his investigations demonstrated that the flies are strongly attracted to these proteins. Steiner (211, 211a, 212a) used as lures for the oriental fruit fly and Mediterranean fruit fly proprietary protein hydrolysates described as "freely soluble enzymatic hydrolysates of primary grown brewers' type nutritional yeast containing free amino acids, polypeptides, and all factors of the vitamin B complex having 70 per cent protein and 6 per cent amino nitrogen," and several other enzymatic hydrolysates, as well as lower cost acid hydrolysates of corn protein. By incorporating these lures with fast acting insecticides, such as Metacide (a formulation of methyl parathion and parathion), parathion, and malathion, the resulting baits attracted flies for distances of 50 feet and when applied as a spray were effective for seven days under field conditions.

In South Africa where the Natal fly, *Pterandrus rosa* (Karsch), is the dominant species of fruit fly and the Mediterranean fruit fly is of secondary importance, Georgala (88) found that the bait developed in Hawaii and used

in the fruit fly eradication program in Florida resulted in higher initial effectiveness in attracting both species than baits containing sugar. The formula contained proprietary hydrolysate known as "Staley's Sauce No. 7" and wettable powder malathion. Staley's sauce was most attractive during the four to six hours immediately following application but the flies were attracted with decreasing effectiveness for about a week. Although Mediterranean fruit fly populations were lower throughout the studies, the results of Staley's sauce baits followed a pattern similar to that of the Natal fly.

Malathion was found, in Hawaii, Florida, and South Africa, to be sufficiently rapid in its toxicity to be a suitable insecticide for combination with Staley's sauce as a toxic bait for these flies. The effectiveness of this bait was decreased by rain and was less efficient during cool or overcast days.

The unusual attractiveness of methyl eugenol to male oriental fruit flies for as much as one-half mile against an eight mile per hour wind (210) is one of the striking examples of insect chemotropisms. Hall *et al.* (104) reported that methyl eugenol is sought out and so greedily devoured by male oriental fruit flies that they will, if permitted, engorge themselves until they die. These workers found that 2-alkoxy-3-ethoxybenzaldehyde attracts both sexes of oriental fruit flies and males have the ability to detect readily dialkoxybenzenes. They are especially attracted to aromatic molecules containing one or more methoxy groups, such as the following:



Each of these methoxybenzenes has a different odor, yet the insect responds to all of them.

Large scale experiments were set up in Hawaii to evaluate the feasibility of using methyl eugenol-Pyrolan baits for control of the oriental fruit fly. Steiner & Lee (212) concluded that feeding stations impregnated at monthly intervals with 30 ml. of methyl eugenol containing 3 per cent Pyrolan and distributed at the rate of 30 or more per square mile are capable of providing substantial control of the oriental fruit fly at a cost of about 35 cents per

acre. Failure to obtain complete control was attributable to migration of fertile females into the treated areas or to the effect of trade winds in preventing adequate dispersal of the attractive odors.

Ripley & Hepburn (194) reported angelica root oil to be moderately attractive to the Natal fruit fly but they considered it less promising than other attractants and apparently did not evaluate it against the Mediterranean fruit fly. As a result of an extensive screening program of possible fruit fly lures at the United States Department of Agriculture fruit fly laboratory in Honolulu, where spot and olfactometer tests were used, a few essential oils were found to be attractive to the Mediterranean fruit fly (213). Of these oils, virgin angelica seed and root oils from *Archangelica officinalis* Hoffmann were found to be outstanding attractants for the males. This discovery has been a vital factor in the progress made toward the eradication of this fly in Florida. During the eradication campaign, the angelica seed oil obtained was variable in composition, freshly prepared samples being less attractive than aged ones, and adulteration was difficult to detect.

Hall *et al.* (104) synthesized at the United States Department of Agriculture laboratory at Beltsville, Maryland, a series of esters of 6-methyl-3-cyclohexene-1-carboxylic acid, some of which were found in tests in Hawaii to be highly effective lures for male Mediterranean fruit flies. In order of increasing effectiveness, they were the propyl, isopropyl, and secondary butyl esters. Field data indicated that the secondary butyl ester is longer lasting than the average sample of angelica seed oil.

The possibility of sterilizing male fruit flies by irradiation and of reducing field populations by their mass liberation is being investigated by the United States Department of Agriculture laboratories at Hawaii and Mexico City. The studies indicate that male oriental fruit flies, Mediterranean fruit flies (37a), and Mexican fruit flies, *Anastrepha ludens* (Loew) (225a), are sensitive to irradiation. The usefulness of this technique in the control of fruit flies will depend on the solution of several technical difficulties.

Adequate and detailed studies on the weather and climatic factors which may delimit the potential distribution of a pest in an uninfested area, whether under quarantine or not, have been far too infrequent, no doubt due to the lack of funds and equipment. By means of bioclimatic cabinets financed by joint United States Department of Agriculture and University of California funds, Messenger & Flitters (161) were able to study the influence of the climate in 18 selected locations in the United States on the biology and development of the oriental fruit fly, the Mediterranean fruit fly, and the melon fly, *Dacus cucurbitae* Coquillett. The cabinets were designed to control temperatures and humidities corresponding with pre-determined patterns which permitted continuous variation, from hour to hour, with diurnal and seasonal fluctuations as occur in nature. Messenger & Flitters found certain stages in the life cycles of the flies more resistant to cold, heat, or drought, thus preserving the population through short periods

of unfavorable weather. On the basis of these findings, the climate of a potential fruit fly area might be divided into three divisions according to the indicated ability of the fruit fly populations to survive throughout the year, namely, favorable, marginal, and unfavorable. Favorable districts had no more than a 60-day period during the year with average temperatures below 57° F. Here continuous development and reproduction, and therefore a steady build-up of successive generations, is probable. Within the United States, southern Texas, Louisiana, and central and southern Florida have such climates. Marginal districts have more than a 60-day and less than a 90-day period below 57° F. Here self-sufficient populations may develop if adult flies or pupae are able to bridge the unfavorable periods. The unfavorable climatic areas may support a population during part of the year but not on a self-sustaining basis throughout the year.

Significant contributions have been made in the treatment of citrus fruits and, if properly utilized, can assure that citrus shipped from infested to uninfested areas is free of the egg or larval stages of fruit flies without adversely affecting the market value of the fruit. Sinclair & Lindgren (203) showed that the vapor-heat sterilization, which was suggested earlier (109) as a means of eradicating fruit flies in fruit for shipment, produced injury or adversely affected fruit quality and even resulted in off-flavored fruit. Of 53 fumigants evaluated against third-instar larvae of the oriental fruit fly, chloroacetonitrile, methyl iodide, methyl thiocyanate, 1,brom-2-chloroethane, ethylene dibromide, 1,3-dichloropropane, and the ethyl ester of chloroacetic acid were the most toxic (10). In studies by Lindgren & Sinclair (152) citrus fruits were not injured upon fumigation with ethylene dibromide, ethylene chlorobromide, or methyl bromide at a dosage of 1 lb. of fumigant per 1000 cubic feet for an exposure period of two hours. In commercial fumigation, however, Sinclair & Lindgren (202) found that fruit and packing materials absorbed much of the gas, thus reducing the concentration remaining for insect control, and they therefore suggested that when fruit is fumigated it should be treated without packing materials or the dosages recommended for fruit only should not be used in fumigating fruit packed or wrapped for shipment.

Biological studies on the Mediterranean fruit fly in various areas of the world have indicated that the degree of injury to citrus is largely dependent on the citrus variety, the yearly sequence of host plants available to the flies, and the prevailing weather conditions during the period fruit is most susceptible to attack. Several workers have suggested disrupting the annual cycle of generations by isolating citrus areas from other susceptible crops and separating early and mid-season varieties from late varieties (83, 159, 196, 225). The mature fruit is more susceptible to attack than the immature fruit which frequently accounts for late season attacks. The larvae in the immature fruit are killed by the gummy secretion of the wounded tissue and the natural oils of the fruit (157, 195). The fly infestations on citrus have been observed to be more severe under high humidity conditions (93, 157,

195). Hanna (106) observed that dry climate conditions favor the rapid drying of the rind around the oviposition puncture, which prevents the larvae from penetrating its walls, and suggested that conditions of humidity unfavorable to the larvae can be produced by spacing the trees far apart, providing good drainage, and watering the trees sparingly.

References to the use of the newer organic insecticides for control of the oriental fruit fly and the Mediterranean fruit fly are too numerous and repetitious to list in this review. DDT, chlordane, dieldrin, lindane, toxaphene, methoxychlor, parathion, malathion, *O*-ethyl *O*-*p*-nitrophenyl phenylphosphonothioate (EPN), and Diazinon [*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothiate] have all been reported as effective. The most efficient or most useful insecticide appears to depend on the conditions under which applications are made. DDT appears to be the material most extensively used; however, malathion plus protein hydrolysate and granular dieldrin were found most applicable to the situations encountered in the successful eradication of the Mediterranean fruit fly in Florida (52).

MITES

The citrus rust or Maori mite, *Phyllocoptruta oleivora* (Ashmead), is probably the most widespread mite pest of citrus; however, experimental studies on its biology and control during the past decade have been relatively meager. The need has perhaps not been critical because of earlier outstanding studies by Yothers (237) and the effective and economical control usually obtained with sulfur applications. More recent evidence that sulfur may be deleterious to some parasites (16, 223) and the difficulties involved in safely using sulfur and petroleum oil in the pest control program has stimulated interest in other acaricides. Chlorobenzilate (ethyl 4,4'-dichlorobenzilate) was found to be more effective than sulfur in California (131), and evidence accumulated against its lack of serious effects on the insect parasites and predators (49), factors which have no doubt been important in the change from sulfur to Chlorobenzilate as the main treatment used to control the citrus rust mite in California.

The discovery by Fisher (79) that the fungicide zineb, zinc ethylenebis(dithiocarbamate), results in effective control of citrus rust mite as well as fungus diseases, led to its adoption as the major acaricide for citrus rust mite control in Florida. Zineb and Chlorobenzilate can advantageously be incorporated with petroleum oil sprays thus presenting the opportunity of decreasing the total number of spray applications per year (136).

Guthion [*O,O*-dimethyl *S*-(4-oxo-3*H*-1,2,3-benzotriazine-3-methyl) phosphorodithioate], Trithion [*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate], (124), and Diazinon (219) have resulted in effective control of citrus rust mite in experimental studies. As these materials have also shown promise in control of insect pests of citrus, they may serve as multipurpose treatments.

The citrus red mite, *Panonychus citri* (McGregor), has been increasing as a pest in several major citrus producing areas, notably central California and South Africa. This increase has been attributed by some observers to the decreased predator and parasite populations resulting from the use of chlorinated hydrocarbon or organic phosphorus insecticides for insect control, while others feel it can be accounted for by the reduction in mite control obtained by these materials as compared to the petroleum oil sprays. It is interesting to note that a similar increase of populations of this pest in the citrus districts of Riverside and San Bernardino Counties of California occurred during the early 1930's. This was attributed by some observers to the reductions of natural enemies through the rather general replacement of HCN fumigation by petroleum oil sprays (19).

Changes in citrus cultural and pest control practices and other factors may also have a strong influence on citrus red mite population trends. Henderson and co-workers (116) and Griffiths & Fisher (95) have accumulated evidence of increased populations of this and other mites by greater use of mineral deficiency sprays. Fleschner (81) laboriously kept field trees free of the natural enemies of citrus red mite and studied the host resistance under this condition and concluded that host resistance of citrus plants to citrus red mite attack ranges from almost complete immunity to marked susceptibility. There are indications that deposits of field dust and of zinc and DDT spray materials lower the host plant resistance and that the effect of DDT lasts some time after application, the effects being translocated from older to younger leaves.

The possibility that citrus red mites have become more adapted to the warmer and drier conditions of the interior valleys of California has been suggested; however, Munger (168) has reared mites from coastal and interior citrus areas under various constant and fluctuating temperature and humidity conditions and found no difference in response.

It appears, therefore, that the development of injurious citrus red mite populations in new citrus districts is not the result of a single change in pest control practices but probably an accumulation of factors favoring mite development.

Fluctuations in citrus red mite during the year and from one season to another have been studied in Florida by Pratt & Thompson (180), who found that populations are inversely correlated with rainfall. Pratt (178) reports that for severe citrus red mite outbreaks to occur in Florida, there must be a cold December and a dry winter and spring. Jeppson *et al.* (128, 129), working in California, found that periods of high temperature and low humidity caused a correlated reduction in mite populations and these conditions appeared to increase the resistance of maturing, or even mature, leaves to mites. The effects varied from district to district depending on the age of the newest growth cycle at the time the adverse conditions occurred, the citrus variety grown, and the duration of the unfavorable periods.

Munger was able to rear citrus red mite in the laboratory by filtering

the air of the rearing room by means of carbon filters (166). His laboratory studies (167) indicated that the most ideal temperature and relative humidity conditions for rearing the citrus red mite in the laboratory were 75° F. and 65 per cent, respectively. A constant temperature of 95° F. with 75 per cent relative humidity produced less adverse effects on the mite colonies than 95° F. and humidities as low as 10 per cent. Under fluctuating temperature conditions similar to the more extreme summer conditions occurring in the San Bernardino County citrus district of California, high humidities again produced more adverse effects on the mite colonies than when relative humidities were kept at 10 per cent.

Petroleum oil is the most extensively used material for the control of citrus red mite either primarily by applications made for this purpose or indirectly by applications made to control scale insects. Riehl & Jeppson, (188) working with narrow-cut fractions of a distinctly naphthenic- and a distinctly paraffinic-type petroleum oil, found the efficiency of petroleum fractions may be increased by proper selection for composition and molecular weight, namely that paraffinic composition is more efficient than naphthenic composition, and that the critical value of molecular weight for highly paraffinic petroleum oils occurs at approximately 340 M. Increased leaf drop, however, indicated that adverse physiological responses are more evident with the higher molecular weight oils.

During the past decade a number of nonoil acaricides has been developed and used for control of citrus red mite. In California, 4,6-dinitro-*o*-cyclohexyl phenol N,N-dicyclohexylamine salt (DN-111) was largely replaced by Neotran [bis(*p*-chlorophenoxy)methane] which is less phytotoxic and more effective under cool weather conditions (120, 121). Ovex (*p*-chlorophenyl-*p*-chlorobenzenesulfonate) was found to produce control improved over previous materials and, therefore, was extensively used either alone when mite populations were low at treatment time or in combination with Aramite [2-(*p*-*tert*-butylphenoxy)-1-methylethyl 2-chloroethyl sulfite] to more rapidly reduce high adult mite populations (122, 125). Demeton was found useful in control of *P. citri* but varied in effectiveness according to seasonal conditions (130, 135). It was used for two years in districts where mites had developed resistance to ovex before it also succumbed to resistance, and laboratory and field tests showed that mites resistant to demeton were also resistant to Trithion, Hercules AC-528 [2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethylphosphorodithioate)], ethion (*O,O,O',O'*-tetraethyl *S,S'* methylene biphosphorodithioate) and other effective organic phosphorus insecticides (133). The use of Aramite has been restricted by tolerance limitations and therefore the newly developed Kelthane [1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethanol] (127) has become the main nonoil material for control of citrus red mite in districts where mites are resistant to phosphates and ovex.

In Florida, DN-111, Neotran, ovex, EPN (*O*-ethyl *O-p*-nitrophenyl phenylphosphonothioate), Chlorobenzide, Isolan [dimethyl 5-(1-isopropyl-

3-methylpyrazolyl) carbamate], demeton, schradan, and Aramite have all been reported as giving satisfactory control of citrus red mite under experimental conditions (138, 141). Dinitrocyclohexylphenol has remained the most extensively used nonoil material for control of this mite with other acaricides being limited to summer treatments when this DN-111 often produces injury. Kelthane, Trithion, Tedion (2,4,4',5-tetrachlorodiphenyl sulfone, 80 per cent technical) have been found effective under experimental conditions and may find a place in the pest control program.

The citrus bud mite, *Aceria sheldoni* (Ewing), first discovered in California (22, 77), then in New Zealand (110), appears to have existed in the old world for centuries (126). It has now been recognized in several countries in the Mediterranean area, South Africa, South America, and Hawaii (7, 41, 59, 63, 65, 89, 101, 148, 197). High temperatures accompanied by low humidities result in high mortalities (112, 134) and may be a limiting factor in the distribution of this mite. In California the citrus bud mite has been largely kept under control by spring and fall applications of petroleum oil sprays. Jeppson *et al.* (132) found Chlorobenzilate to be effective and one summer application resulted in annual control (134).

The oriental citrus mite, *Eotetranychus orientalis* (Klein), a well known pest of Israel, India, Egypt-Sudan, Formosa, and other areas, appeared in outbreak proportions in Cyprus during 1956 to 1957 (89). The biology and optimum conditions for development of this pest were worked out by Klapperich (142). Severe infestations of the six spotted mite, *Eotetranychus sexmaculatus* (Riley), occurred in only six out of 19 years in Florida and these outbreak years followed low December temperatures (180). In California this mite is confined to orchards located near the ocean (123). The Texas citrus mite, *Eotetranychus banksi* (McGregor), is a recent addition to the injurious mites of Florida (143). Bitancourt (17) in Brazil and Thompson (220) in Florida have added evidence that Leprosis, or scaly bark disease, of these countries is associated with the presence of *Brevipalpus obovatus* Donnadieu. A related species, *Brevipalpus lewisi* McGregor, has in recent years produced scarring of orange and tangerine fruit in the interior valleys of California (70). Populations of these mites can be effectively reduced by applications of sulfur, Chlorobenzilate, and Kelthane (17, 70).

MISCELLANEOUS INSECT PESTS

Some aphid species may be found in most citrus orchards; however their direct damage is generally limited to the very young growth and, therefore, to relatively short periods during the year. It is commonly believed that the characteristic curled leaves produced by aphid feeding retard the growth of young orange trees, and the effects measured in delay in the time required for a citrus orchard to reach economic production usually justifies the cost of aphid control; however, limited experimental results in California on Valencia orange trees do not substantiate this belief (174). On mature orange trees the value of controlling aphids by chemical applica-

tions has not generally been conceded. Reitz *et al.* (184) studied the effects of aphid populations on mature Hamlin and Temple varieties of orange trees and found that the average fruit size on trees heavily infested with aphids was decreased 4.3 mm. on the Hamlin and 2.9 mm. on the Temple varieties. Yield data did not indicate a significant loss in production; however, the authors concluded that aphid damage may produce measurable decrease in fruit size, some decrease in yield, and effects on size of considerable economic significance. Ortega & Jones (174) studied the effects of aphids on mature Valencia orange trees in California and found no effect on either fruit size or production.

The important discovery by Meneghini (160) that the tristeza virus of citrus trees was transmitted by *Aphis citricidus* (Kirkaldy) has stimulated investigations on the transmission of virus diseases of citrus by aphids. Fernandez Valiela in 1948 (78) reported transmission of the tristeza (root rot) virus by *A. citricidus* (*Paratoxoptera argentinensis* Blanchard). Dickson and co-workers (55, 56, 57) found that the so-called "quick decline virus" (tristeza) of orange trees in California was transmitted by the melon aphid, *Aphis gossypii* Glover. They showed that this aphid was a weak vector of the virus but adequate to account for the spread of the virus in California. McClean (155) demonstrated that the virus disease widely known as stem pitting is transmitted by the black citrus aphid, *A. citricidus*; Hughes & Lister (117) found that this aphid was also responsible for the transmission of lime die back and concluded that these diseases as well as tristeza were caused by a similar virus complex, if not the same virus. Norman & Grant (172) reported transmission of tristeza virus by the green citrus aphid, *Toxoptera aurantii* (Fonscolombe), and by the melon aphid, *A. gossypii* Glover. Other virus diseases (relationship to tristeza unknown to the authors) which have been reported to be transmitted by aphids are grapefruit dimple (26) and lace-bark (78).

Much has been written concerning the activity of ants in cultivating honeydew-producing insects and their role in protecting such pests as the unarmored scales, mealybugs, whiteflies, and aphids from their predators and parasites, thus permitting injurious populations to develop in orchards where pests and natural enemies are normally in biological balance. Effective ant control has been achieved with chlordane, dieldrin, endrin, and Gammexane (1,2,3,4,5,6-hexachlorocyclohexane) (80, 90, 91, 169, 216). These materials were successfully applied as bands around tree trunks and as ground applications in the form of sprays and dusts, depending on the species and orchard situations. Formulation of these materials as granules has permitted their rapid distribution over the ground surface by airplane or by fertilizer-type distributing equipment without subjecting the fruit to insecticide residues (28).

Guillemain & Alibert (100) indicate that two species of ants of the genus *Tapinoma* prevalent in North Africa devour the young shoots, flowers, and fruit as well as protect honeydew-producing insects. Trees may be pro-

ected by fixing glass wool bands impregnated with DDT, white oil, and water around the tree trunks. Working in Argentina citrus orchards, Otamendi (175) was able to destroy 95 per cent of the colonies of leaf cutting ants of the genera *Atta* and *Acromyrmex* by injecting carbon bisulphide into the nests, and, when followed by inspection and treatment of incipient colonies at three to six month intervals after the initial treatment, complete ant control was obtained (8).

For many years citrus growers in the San Joaquin Valley of California have sprayed citrus orchards in the fall of the year with heavy dosages of lime to prevent orange fruit damage from leafhoppers, *Empoasca fabae* (Harris) or *Empoasca solana* DeLong or both. The leafhoppers move into citrus orchards from the native plants and cultivated crops, and their feeding on the fruit produces rind oil spotting. Similar injury by the same species has been reported in Arizona (145) and by *Empoasca decadans* (Paoli) in Sicily (58, 60, 61).

In 1952 a capsid bug, *Distantiella collarti* Schout, was found damaging citrus in the Belgian Congo. It attacks orange, mandarin, and grapefruit, causing lesions just below the young shoots on the twigs, which wither, shed their leaves, or even break at the injured point. A nymph may produce 10 to 20 punctures during a night (51). In Florida *Leptoglossus gonagra* (Fabricius), *Leptoglossus phyllopus* (Linnaeus), *Euthochtha galeator* (Fabricius), and other plant bugs fly into citrus from leguminous or other crops, and their feeding injury causes fruit to drop. *E. galeator* also feeds on tender, succulent twigs in the spring, causing them to wilt and die (98).

The citrus thrips, *Scirtothrips citri* (Moulton), a citrus pest unique to California, has rapidly developed resistance to insecticides. Tartar emetic, reported to be an effective treatment by Boyce & Persing in 1939 (23), was ineffective in some orchards by 1942 (24, 177) and by 1952 resistance had developed in most citrus districts (76). DDT came into extensive use as a treatment for this pest in 1946 (21, 176) and by 1949 resistance was evident in some groves in the San Joaquin Valley (76). In 1952 the effectiveness of dieldrin was detailed by Ewart *et al.* (76) and resistance to this insecticide was reported three years later. The control of this pest with chemicals appears to be a challenging resistance problem.

Natural enemies of whiteflies have been effective in keeping populations of most species from causing economic damage to citrus trees; however, sometimes chemical treatment is necessary to protect citrus from severe injury. Wille reports that the wooly whitefly, *Aleurothrixus floccosus* (Maskell), a serious pest of citrus in Peru since 1953, can best be controlled by several applications of petroleum oil and parathion (233, 234). The citrus blackfly, *Aleurocanthus woglumi* Ashby, a serious citrus pest in Mexico, has more recently caused serious damage in Pakistan (1) and the Seychelles (105). Guicherd (99) reviewed the factors inimical to this pest, namely, rain which destroys the adults, hot sunlight which kills all stages, drought which kills the larvae, wind which causes the destruction of the egg by shaking them off

the trees, insect predators, and fungal and insect parasites. These factors have largely limited the populations of this potentially injurious citrus pest.

The citrus psylla, *Diaphora citri* (Kuwayana), the most serious citrus pest in Punjab, was found by Latif & Khan (146) to be most effectively controlled by applications of Diazinon. Harbans Singh (107) found that endrin was more effective than nicotine sulphate for control of this pest.

Injury to citrus from weevils has been prevented by setting up toxic or mechanical barriers to the adult weevils, thus preventing them from reaching the aerial parts of trees after emerging from their pupal cells in the ground. Branches adjacent to the ground are removed and mechanical barriers constructed around the tree trunk or the soil under the tree, or the lower branches and trunk are treated with a suitable toxicant. Such methods are used to prevent injury by the Fuller rose beetle, *Pantomorus godmani* (Crotch), a sporadic pest in several important citrus areas of the world (53, 62, 113, 118), and the Dicky rice weevil, *Maleuterpes spinipes* Blackburn, in coastal regions in Australia (71). O'Loughlin (173) used DDT-treated wire cages to indicate the time of emergence and density of beetle population; these cages could be used to anticipate the need for foliage treatment and permit the establishment of application schedules. Beetles that bore into the wood of either healthy, diseased, or injured trees are sometimes an important problem (37, 48). Control methods developed so far, although probably adequate, appear to be cumbersome and in most cases impractical.

Cutworms have caused considerable damage to citrus in limited areas of California (3). Many cutworms feed on the cover crop and, therefore, can be controlled by clean cultivation; however, according to Atkins (6) the citrus cutworm adult, *Xylomyges curialis* Grote, deposits its eggs on citrus foliage and its larvae feed entirely on citrus.

Economic losses to citrus from the garden tortrix, *Clepsis peritana* (Clemens), have become progressively greater during the past nine years at which time it was recognized as being distinct from the orange tortrix, *Argyrotaenia citrana* (Fernald) (5).

In 1956 all new growth of orange trees on a limited area of southern California was destroyed by the western tussock moth, *Hemerocampa vestusta* (Boisduval) (4). Atkins found DDT, DDD [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane], and parathion to be among the most effective materials tested against these lepidopterous larvae, and with these materials it is feasible to treat when populations reach economic levels rather than apply preventative applications.

A study of food plants of the citrus leaf miner, *Phyllocnistis citrella* Stainton, in Pakistan by Latif & Yunus (147) indicated that the citron, *Citrus medica*, which has comparatively succulent leaves, was the most susceptible host of this pest and, as it has no great economic importance, should be removed from citrus orchards. Lemons should not be planted as a hedge around gardens for they provide an undisturbed breeding ground for this miner.

Premature fruit drop had largely been attributed by Philippine Island growers to a citrus rind borer, *Prays endocarpa* Meyrick, until Viado *et al.* (230) showed that this pest was not the primary cause of the fruit drop. Control measures are necessary, however, because the insect produces unsightly swellings on the rind of mature fruit, and endrin and EPN (*O*-ethyl *O*-*p*-nitrophenyl phenylphosphorothioate) resulted in most effective control on Szinkom mandarin and Siamese pummelo, respectively. Bajpai (9) reports that fruit-piercing moths, *Othreis fullonia* (Clemens) and *Othreis materna* (Linnaeus), which cause fruit drop of a mandarin variety of citrus in the state of Madhya Pradesh, India, could be controlled by a bait mixture of molasses, vinegar, water, and lead arsenate.

Studies by Gupta (102) on the biology of the citrus leaf roller, *Tonica sisyphi* Stainton, a pest of citrus in India and Ceylon, indicate that hot weather is unfavorable for its development and populations increase during the rainy periods.

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PESTICIDES IN RELATION TO PUBLIC HEALTH¹

BY WAYLAND J. HAYES, JR.

Communicable Disease Center, Bureau of State Services, Public Health Service, United States Department of Health, Education, and Welfare, Savannah, Georgia

Pesticides promote health directly through the control of vector-borne diseases and indirectly through increased and improved agricultural production. On the contrary, the extensive use of any biologically active chemical implies a potential hazard which has to be evaluated.

The public health aspects of these valuable, but potentially dangerous, economic poisons have been ably reviewed, sometimes at greater length than is permitted here (6, 8, 53). The facts have changed very little. This review attempts to present some results of recent research together with enough narrative to supply continuity.

BENEFITS TO HEALTH

Most people at least have heard of the revolution which DDT produced in disease control. Other compounds including dieldrin, benzene hexachloride, and chlordane now have made contributions chiefly in the control of adult mosquitoes and the triatomid bug, *Rhodnius prolixus* Stål, a vector of American trypanosomiasis. Lindane has been used for control of human body lice, especially where these vectors of typhus and relapsing fever have developed resistance to DDT. Diazinon [*O,O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate] and malathion have been used for control of adult mosquitoes, and Diazinon, parathion, and malathion have been used against mosquito larvae. Most of these compounds have been used against flies; however, these materials are largely substitutes for DDT and their method of use has remained generally imitative so far as disease control is concerned. Even now, DDT is used in vector control more frequently than all other insecticides combined.

The contribution of DDT to the control of malaria, filariasis, dengue, urban yellow fever, virus encephalitis, louse-borne typhus, louse-borne relapsing fever, trench fever, plague, murine typhus, bacillary and amebic dysentery and diarrhea, leishmaniasis, bartonellosis, onchocerciasis, sandfly fever, trypanosomiasis, yaws, infectious conjunctivitis, cholera, Chagas' disease, scrub typhus, scabies, rickettsialpox, tick-borne relapsing fever, Rocky Mountain spotted fever, and tularemia was the subject of a 251-page review by Simmons published in 1959 (95). The earliest relevant papers were concerned with measurements of vector control in treated and in comparable, untreated places. It required longer to collect epidemiologic evidence and, thus, demonstrate that disease control had been achieved, but this now has been done in a most impressive way. A single example (Figure 1) must suffice.

¹ The survey of the literature pertaining to this review was concluded in June, 1959.

In his review, Simmons points out that malaria control, brought about by DDT, has been a factor in permitting some increase of population and a great increase in agricultural productivity in certain areas of Madagascar and India. Such a real improvement in the welfare of the community may be expected to follow improvement in the health of the members. However, this social change is more difficult to measure and document than the spleen index or parasite rate.

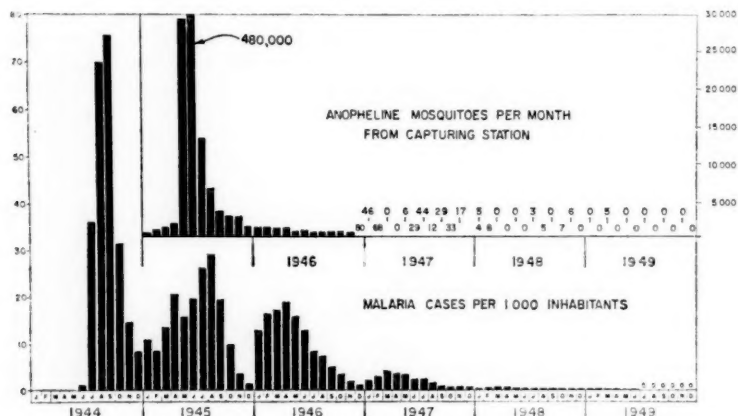


FIG. 1. Number of anopheline mosquitoes per month from capturing stations in Latina Province, Italy, and number of malaria cases per 1000 inhabitants in the same Province following spraying with DDT from June, 1945, onward [modified from Simmons (95)].

There has been such outstanding success already in disease control through the use of DDT and other chemicals that the eradication of malaria and urban yellow fever is now being attempted in many countries with the active support of the World Health Organization, the Pan American Sanitary Bureau, United Nations Children's Fund (UNICEF), International Co-operation Administration, and other international health agencies. In some countries, very much improved epidemiologic surveillance will be required if eradication is to be achieved. Drugs may be useful in the public health aspects of disease control as well as in the treatment of the individual case. However, there is still much room for improvement in vector control. This would be true even in the absence of resistance. It is much too early to evaluate the suggestion by Mathis *et al.* (74) that DDVP (2,2-dichlorovinyl dimethyl phosphate), Diazinon, malathion, and perhaps other organic phosphorus compounds which produce insecticidally effective vapors may be used for control of mosquitoes in dwellings. Many technical problems remain to be

solved. However, the first really new idea in vector control since the introduction of DDT may have been suggested.

Even in a country with agricultural surpluses, we cannot ignore the contribution which insecticides make to our food and shelter. If effective insect control and other factors did not make it possible for farmers to produce food at a higher rate than formerly, we simply would require more farmers and more cultivated land or face starvation. In spite of the progress, it is estimated that insects destroy or spoil over 10 per cent of the total crop in this country and that really thorough control might show this to be an underestimate (32). The situation in the tropics is far worse: malnutrition frequently is present, insects present a more severe problem, and less progress has been made in their control. Decker (32) has pointed out that major livestock enterprise was delayed 200 years even in our own southeastern states by uncontrolled arthropods, notably ticks and screwworm flies. It is thought that the thorough application of the best present methods would reduce agricultural losses caused by insects to one-half. The control of agricultural insects certainly must go hand-in-hand with the control of disease vectors if underdeveloped countries are to approach the achievement of health and low infant mortality now enjoyed by the countries more advanced in technology.

PRODUCTION AND USE

In view of the great benefits from insecticides, it is no wonder that their production has increased. It is estimated that pesticides reached a value of \$274,894,750 at the manufacturer's level in 1956 (93). It is estimated that insecticides account for 36 per cent of the cost; fungicides, 32 per cent; herbicides, 13 per cent; and miticides, soil fumigants, space fumigants, and rodenticides and miscellaneous economic poisons, 8, 5, 4, and 2 per cent, respectively (41, 42, 43).

Over 120,000,000 pounds of technical DDT alone have been produced annually for several years. The annual production of synthetic organic pesticides in the United States has increased steadily, reaching a level of 569,927,000 pounds in 1956 (93). Not only has production of a wide range of pesticides increased, but, as shown in Figure 2, this increase is expected to continue.

Another indication of the growing magnitude and variety of production is the registration of economic poisons under the Federal Insecticide, Fungicide, and Rodenticide Act as shown in Figure 3. Of the cumulative total of 90,816 registrations through June, 1958, of which 51,626 were original and 39,190 supplementary, only 8586 were cancelled voluntarily from 1953 onward (109).

To be sure, most of the registered formulations are mixtures with relatively minor variations in combinations. It has been stated (81) that only about 200 basic chemicals are registered for use in pesticide formulations and that only a small proportion of these account for the majority of the total value. Thus, it is said that there are only fifteen to eighteen major insecti-

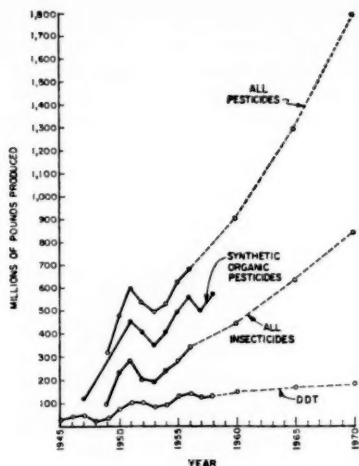


FIG. 2. United States production and predicted production of DDT, all insecticides, synthetic organic pesticides, and all pesticides. [Data from Commodity Stabilization Service, U. S. Department of Agriculture and estimates from Fisher, 1956 (41, 42, 43) and President's Materials Policy Commission, 1952.]

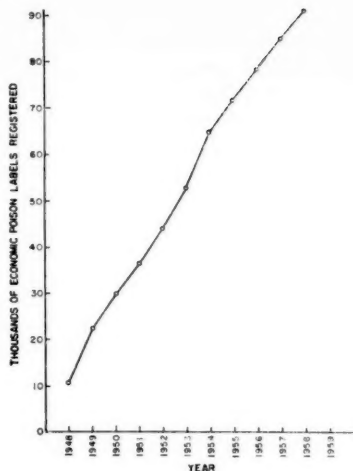


FIG. 3. Total number of economic poison labels registered under the Federal Insecticide, Fungicide, and Rodenticide Act [From Ward (109)]. Includes 51,626 original and 39,190 supplemental registrations as of June 30, 1958.

cides, ten to fifteen fungicides, four or five soil fumigants, eight to ten herbicides, and four or five rodenticides. Although this is undoubtedly true from an economic standpoint, it is necessary to take into account toxicologically all of the registered basic chemicals and a much larger number of candidate materials.

Furthermore, as new insecticides are introduced, they supplement, but do not replace, older ones. This is illustrated by Figure 4, which shows the production of two arsenicals contrasted with the production of DDT and BHC (1, 2, 3, 4, 5, 6-hexachlorocyclohexane). Again, although DDT, the first synthetic insecticide introduced, is still made in greater tonnage than any other, it is estimated that the phosphorus compounds now represent between 15 and 20 per cent of the total value of insecticides produced in the United States (81).

Paralleling the increase in pesticides, there has been an increase in the number and variety of their uses and in the methods of their application. Crops are treated which formerly were left unprotected. The expansion of suburbs has been associated with increased use of pesticides by amateurs for lawns and small flower or vegetable gardens. Figure 5 shows the trend in

the number of acres of crop land in California treated by aircraft. Another relatively new method of application involves the use of low volume, high concentration sprays applied by tractor-drawn sprayers.

MAGNITUDE OF THE TOXICOLOGY PROBLEM

Some indication of the interest in the potential hazard of pesticides is indicated by the number of laboratories investigating the toxicity of these compounds. Thirty-six of these laboratories and tabular data on them have been listed by the World Health Organization (111).

The hazard of a given compound is the danger which it presents; the toxicity of a compound is its inherent ability to injure living organisms. Hazard can be measured only in relation to practical conditions, although it can be predicted, to a limited extent, from toxicity. Conversely, toxicity may be guessed from the results of accidents; but, unless the dosage involved in such accidents is known accurately, toxicity can be measured only under standardized experimental conditions. Chemical structure largely determines the absorbability, toxicity, and metabolic fate of a chemical, but the way the material is used determines whether it ever will be available for absorption in the first place. The toxicity of each compound usually varies for different species and different ages and may vary for the two sexes. The toxicity of a

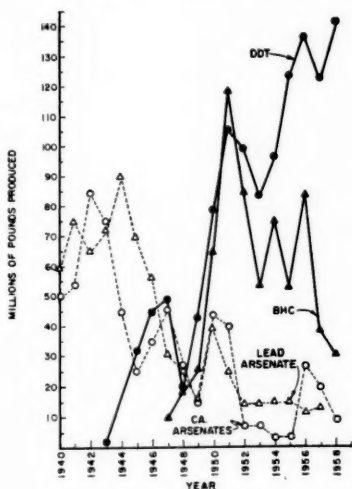


FIG. 4. United States production of two arsenical insecticides and two synthetic insecticides. (Based on U. S. Tariff Commission and Bureau of Census figures.)

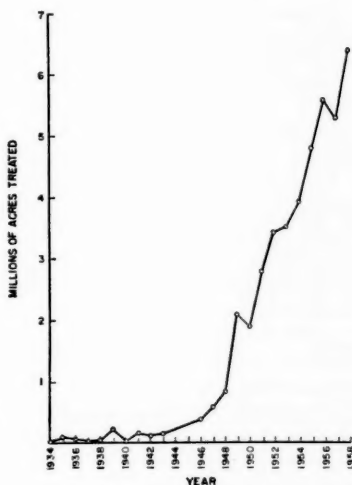


FIG. 5. Area of land in California treated with pesticides in successive years. (Based on Annual Reports, California Department of Agriculture.)

compound may be influenced by its stability and particle size, the presence of solvents or other adjuvants, the nutrition of the subject, the timing of application, and other factors. Chronic toxicity may be determined by the storage of a poison or its metabolites in the tissues, or by the accumulation of some biochemical or morphological tissue injury, or both, or by both storage and tissue injury. The hazard presented by a compound depends on all the aspects of its toxicity and also on the method of use, the degree of odor or other warning it offers, the education of those who use it or contact it indirectly, and other subtle factors.

TABLE I
DEATHS FROM SELECTED GROUPS OF CAUSES AND CRUDE DEATH RATES
PER 100,000 POPULATION, UNITED STATES, 1956*

Cause	Number	Rate†
All causes	1,564,476	935.4
Malignant neoplasms	247,357	147.9
Cardiovascular-renal diseases	854,152	510.7
Alcoholism and other drug addiction	2,161	1.3
Accidents	94,780	56.7
Motor vehicle	39,628	23.7
Poisoning by gases and vapors	1,213	0.7
Poisoning by solids and liquids	1,422	0.9
All other accidents	52,517	31.3

* U. S. Public Health Serv. Vital Statist. U. S., 1956 (1958).

† Per 100,000 population.

Hazards associated with insecticides and other pesticides include the following. (A) Hazards of manufacture or formulation. (B) Hazards of mixing or application. (C) Hazards to persons not directly or intentionally associated with chemicals including: (a) children and irresponsible adults, (b) persons whose food bears residues of chemicals, and (c) persons exposed incidentally or environmentally.

The danger from pesticides may be judged from mortality statistics, morbidity records, and special surveys. The deaths from selected groups of causes and the crude death rates per 100,000 population in the United States during 1956 are shown in Table I. Most poisoning by pesticides falls in the category of accidental poisoning by solids and liquids. Poisoning by fumigants forms only a small proportion of the category of accidental poisoning by gases and vapors. It may be seen that all kinds of fatal accidental poisoning constitute only a small proportion of deaths from accidents generally and an even smaller proportion of deaths from all causes.

Table II shows the total accidental deaths in the United States caused by chemicals for the period 1939 to 1956. It may be seen that the number of

cases attributed to solids and liquids has increased slightly during the period but there has been little change in the rate of poisoning because of population growth. There has been no increase in the rate of poisoning following the commercial introduction of DDT in 1946 and the introduction of other new materials in subsequent years. Rather, it would seem that a gradual improvement in the record still is taking place.

TABLE II
TOTAL ACCIDENTAL DEATHS IN THE UNITED STATES CAUSED BY
CHEMICALS AND RATE PER 100,000 POPULATION*

Year	Gases and vapors		Solids and liquids	
	Number	Rate	Number	Rate
1939	1,396	1.1	1,371	1.0
1940	1,562	1.2	1,324	1.0
1941	1,464	1.1	1,191	0.9
1942	1,694	1.3	1,193	0.9
1943	2,038	1.5	1,254	0.9
1944	1,907	1.4	1,381	1.0
1945	2,131	1.6	1,532	1.2
1946	1,874	1.3	1,536	1.1
1947	1,938	1.4	1,504	1.0
1948	2,002	1.4	1,436	1.0
1949	1,617	1.1	1,634	1.1
1950	1,769	1.2	1,584	1.1
1951	1,627	1.1	1,497	1.0
1952	1,397	0.9	1,440	0.9
1953	1,223	0.8	1,391	0.9
1954	1,223	0.8	1,339	0.8
1955	1,163	0.7	1,431	0.9
1956	1,213	0.7	1,422	0.9

* U. S. Public Health Serv. Vital Statist. U. S., 1939-1956 (1940-1958).

The classification "Other Acute Poisonings" used around the turn of the century essentially is comparable to a combination of the three classifications used today: "Accidental Poisoning by Solid and Liquid Substances," "Accidental Poisoning by Noxious Foodstuffs," and "Accidents Caused by Bites and Stings of Venomous Animals and Insects." In 1956, the latter three categories carried a combined rate of 0.9 per 100,000 population; the rate for the former classification for the death registration states of 1900 was 4.2 per 100,000. During the following decade, this figure rose to 5.2 in 1903 and then declined to 3.4 in 1909. As shown by Table III, the gradual decline in the number of deaths caused by the older pesticides (only two of which are

TABLE III

NUMBER OF ACCIDENTAL DEATHS IN THE UNITED STATES
ATTRIBUTABLE TO SELECTED CAUSES*

Year	Arsenic and related compounds	Nux vomica and strychnine	Barbituric acid and derivatives
1939	109	82	201
1940	73	67	246
1941	70	40	232
1942	71	50	197
1943	85	54	226
1944	77	54	270
1945	58	52	392
1946	63	45	436
1947	48	37	418
1948	63	30	419
1949	57	22	466
1950	58	23	409
1951	57	16	363
1952	49	24	327
1953	52	21	337
1954	37	14	345
1955	41	17	411
1956	58	7	323

* U. S. Public Health Serv. Vital Statist. U. S., 1939-1956 (1940-1958).

listed) is counterbalanced by an increase in accidents involving sleeping pills, for example.

A careful study of deaths attributed to accidental poisoning in the United States in 1956 has been made by Short (94). Deaths caused by solid and liquid pesticides constituted 9.8 per cent of those caused by solid and liquid substances generally. This finding is similar to that of Conley (26), who reported that 7.8 to 12.8 per cent of poisoning cases each year from 1946 through 1955 were caused by pesticides. In the United States during 1956, 152 deaths were caused by pesticides as follows: arsenic, 54; phosphorus, 21; thallium, 8; mercury, 2; nicotine, 4; strychnine, 3; rotenone, 1; camphor, 1; parathion, 11; Diazinon, 2; demeton, 1; TEPP (tetraethyl pyrophosphate), 1; unidentified organic phosphorus insecticide, 1; endrin, 3; chlordane, 3; lindane, 2; aldrin, 1; dieldrin, 1; combinations of chlorinated hydrocarbon insecticides, 2; other specified pesticides, 17; and unspecified pesticides, 13.

It is sometimes assumed that most deaths caused by lead and arsenic are associated with pesticides. This was true in 1956 for arsenic but not for lead. Of 62 presumably accidental deaths involving arsenic (including four originally not so classified), 54 were caused by economic poisons, one was caused by a mercurial medication, and seven were really suicides. On the contrary,

not a single case of lead poisoning was associated with a pesticide. Most of the lead cases were associated with paint gnawed from surfaces by infants. It was true that most deaths caused by pesticides were assigned to the category "other and unspecified solid and liquid substances;" of 144 pesticide cases attributed to solids and liquids during 1956, 86 fell in this category.

It is of interest to note that 104 of the 152 deaths caused by all pesticides in 1956 were caused by compounds introduced before DDT. Only 35 deaths definitely were associated with the new synthetic compounds, although some of the 13 other deaths caused by unidentified pesticides doubtlessly were caused by synthetics. During 1956, children up through the age of nine accounted for 94 deaths from pesticides (62 per cent); 78, or just over half of the total number, were children three years old or younger. In 1956 and other recent years also, the older compounds remained the chief cause of fatal poisoning by pesticides and children remained the chief victims.

It frequently is estimated that there are about 100 nonfatal accidents for every fatal one. The ratio may be even higher if one includes as cases those episodes where exposure is observed or suspected without the development of any characteristic symptoms. Among a total of 2407 hospitalized poisoning cases reported by the American Red Cross for one important city, the ratio of nonfatal to fatal cases varied from 25:1 to 115:1 for the separate years 1951 through 1958 and averaged 50:1. By contrast, among cases reported to poison control centers, the rate of hospitalization was less than 10 per cent, and the ratio of nonfatal to fatal cases is 750:1. Approximately 70 per cent of cases reported to poison control centers were asymptomatic (20).

Among the 2407 hospitalized cases mentioned above, the proportion of nonfatal and fatal cases associated with pesticides was 5.8 and 4.3 per cent, respectively. These percentages are of slightly lower magnitude than those given earlier for fatal cases associated with pesticides, perhaps because the values come from an urban area. In one report of 579 cases reflecting considerable rural experience (87), only the more serious cases were recorded (ratio of 13 nonfatal to 1 fatal), and the proportion of nonfatal and fatal cases caused by pesticides was 10.4 and 8.9 per cent, respectively.

A number of surveys of the incidence of poisoning have been carried out, sometimes as the result of local complaints that insecticides were the cause of illness in the persons who were exposed unintentionally and only slightly (45, 50, 60, 90, 98). In each instance, cases of acute poisoning were found among those heavily exposed and reported in the papers cited or separately. The degree of illness corresponded, as nearly as could be judged, to the degree of exposure. Cases of mild poisoning resembled severe cases except in degree. Men who had extensive occupational exposure to parathion frequently showed some depression of blood cholinesterase activity, even though they remained well. However, in no instance were insecticides found to be a cause of chronic effects or a contributing cause of asthma, influenza, or any other of a wide range of diseases which some have attributed to DDT or to pesticides generally. It has been possible to measure the excretion of *p*-nitro-

phenol in the urine of people whose only exposure to parathion resulted from their living near treated orchards (40). However, the occurrence of even mild illness always was associated with greater exposure and the excretion of the parathion derivative at a much higher rate than that encountered by these environmentally exposed persons.

METHODS OF STUDY AND CLINICAL RESULTS

By necessity, first estimates of the safety of a pesticide must be based on its toxicity to animals. The evaluation of chemicals which may occur in trace quantities in food is based exclusively on animal tests, analytic methods, and

TABLE IV
RELATIONSHIP BETWEEN TOXICITY AND SAFETY OF PESTICIDES

Compound	LD ₅₀ values in male rats		Kind of exposure associated with systemic poisoning	Greatest severity of observed poisoning
	Oral	Dermal		
DDT	113	2,510	Ingestion	Severe
Lindane	88	1,000	Ingestion	Severe
Dieldrin	46	90	Ingestion and occupational	Severe
Methyl parathion	18	65	Occupational	Mild
Guthion*	13	220	Occupational	Mild
Parathion	13	21	Ingestion and occupational	Severe
Thimet†	2	6	Occupational	Severe
Phosdrin‡	6	5	Occupational	Severe

* *S*-(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-ylmethyl) *O,O*-dimethyl phosphorodithioate.

† *O,O*-diethyl *S*-(ethylthio)methyl phosphorodithioate.

‡ 2-methoxycarbonyl-1-methylvinyl dimethyl phosphate.

the results of residue measurements. The most recent and complete presentation of the tests required by the Food and Drug Administration is that of Lehman *et al.* (71). A discussion of these tests is beyond the scope of this paper, but anyone interested in the scientific aspects of the problem must consider the critical review of Barnes & Denz (7).

New residue tolerances are published in the *Federal Register*. A generally available list complete through March 1, 1959, has been published by the National Agricultural Chemicals Association (86).

There is a useful correlation between occupational hazards based on actual use experience and dermal LD₅₀ values, while the correlation with oral LD₅₀ values is less close. This is illustrated by Table IV from Gaines (49). The prediction of occupational poisoning may be improved somewhat by studying the effect of repeated doses of a pesticide in animals. However, ani-

mal tests serve their best purpose when some idea of the mode of action can be grasped and explored.

Because the toxicity of a chemical differs for different species, it is necessary that a final determination of danger be based on studies of man himself. Such studies may involve cases of accidental poisoning when the dosage is known, cases of occupational exposure when that exposure can be measured and clinical effects studied, and finally, the careful investigation of people who voluntarily receive measured doses according to a predetermined plan. Several insecticides now have been studied in man.

The potential hazard of DDT lies in its possible long-term effects and in the fact that it is stored, even when absorbed in small amounts. Walker *et al.* (108) found that DDT occurred in traces in all of the 18 restaurant meals which they analyzed. The average daily intake of DDT based on all meals—breakfast, lunch, and dinner—was 0.184 mg. The greatest amount of DDT which could have been eaten in the three meals of a day was 0.388 mg. The storage of DDT in human fat was demonstrated early (65, 70).

In view of these facts, Hayes and his colleagues (58) fed volunteers DDT dissolved in oil at doses of 0, 3.5, and 35 mg. per man per day. Because all the men were on an ordinary diet, these dosage levels were chosen as being about 1, 20, and 200 times the ordinary dietary level. Along with four controls, 10 men ate the DDT daily for a year and a few of them for somewhat longer. No clinical effect associated with dosage was detected either by the men themselves or by careful physical examination and laboratory testing. The storage of DDT was measured and a method for accurately measuring the excretion of DDA [bis(chlorophenyl) acetic acid] in the urine was developed (28). About one year was required for the men to approach equilibrium of DDT storage. The storage of DDT in the fat was proportional to dosage. It ranged from 3 to 13 p.p.m. (mean, 7.4 p.p.m.) for all the men at the beginning of the experiment. Following 10 or more months of dosage at 20 times the ordinary dietary level, one man stored recrystallized DDT at a concentration of 90 p.p.m., while two others stored technical DDT at concentrations of 26 and 33 p.p.m. Following the same length of dosage at 200 times the ordinary dietary level, the men stored recrystallized DDT at concentrations of 216 to 466 p.p.m. (mean, 340 p.p.m.) and technical DDT at 101 to 367 p.p.m. (mean, 234 p.p.m.). The difference between recrystallized and technical DDT was statistically significant, but the physiological basis is not fully understood. The study is being repeated with 21 months of dosage and 27 months of further observation with emphasis on measurement of DDA excretion and on the loss of DDT from storage following cessation of dosage.

In the meantime, measurement of DDT storage in the fat of groups of people with different degrees of dietary, environmental, and occupational exposure revealed that those who lived near treated fields show only a trivial increase of DDT in body fat as compared with people in the general population (61). People who eat essentially no meat store significantly less DDT (mean 2.3 p.p.m.) than the general population (mean 4.9 p.p.m.), as one

would predict from the greater average concentration of the compound in animal fat in contrast to other food (108). Recent occupational exposure to DDT in agriculture produced an average storage of 17.1 p.p.m. Heavy occupational exposure produces high storage levels. The highest value reported for man was 648 p.p.m. found in a worker in a formulating plant (58). Orteele (88) examined 40 formulating plant workers and measured the excretion of DDA [bis(chlorophenyl)acetic acid] in their urine. The results indicated that 26 of these men had absorbed DDT at a rate equal to, or higher than, that associated with an oral dose of dissolved DDT at the rate of 35 mg. per man per day. The men had been exposed at this rate for 0.5 to 6.5 years (mean, 3.5 years) but showed no ill effects as judged by medical examinations, their own reports, or their work-attendance records.

The oral toxicities of schradan, dimefox, and parathion in man have been studied by Edson (35, 36, 38). Daily doses of schradan at the rate of 0.013 mg. per kg. per day caused moderate cholinesterase depression but no signs or symptoms. Daily doses of dimefox as great as 0.0020 mg. per kg. per day caused no physiological disturbance and no significant depression of blood cholinesterase. Daily doses at the rate of 0.0034 caused a slight but definite depression of cholinesterase without any symptomatology. In a similar way daily doses of parathion at a rate of 0.021 mg. per kg. per day produced no effect while 0.078 mg. per kg. per day produced a moderate depression of blood cholinesterase without clinical evidence of injury.

The oral toxicity of dinitrophenol was studied by Harvey *et al.* (57) who found that two of five men given 75 mg. of DNOC (4,6-dinitro-*o*-cresol) daily for five or more days developed headache, lassitude, and malaise after five and seven days. The symptoms corresponded with blood levels of 20 p.p.m. and with peaks of 40 and 48 p.p.m. In practice, it seems wise to discontinue the exposure of workers when their blood level reaches 15 p.p.m. and obligatory to do so when the blood level reaches 25 p.p.m. (37).

The toxicity of aerosols containing malathion was investigated by Golz (52). Groups of four men each received 84 one-hour exposures in 42 consecutive days at dosages of 0, 0.15, 0.6, and 2.4 gm. of malathion per 1000 cu. ft. of air. At no time did the men experience any cholinergic symptoms or signs, nor was there any significant or dose-related change in blood cholinesterase. The highest dosage, which was far greater than anything which would result from practical use, was moderately irritating to the nose and conjunctiva.

The mechanism of action of TEPP (tetraethyl pyrophosphate) on the eye was studied by Upholt *et al.* (99). It was found that instillation of two drops of a 0.1 per cent solution in peanut oil causes maximal miosis, a decrease in light perception, and an increased depth of focus in the eye. When the application was unilateral (but not when the application was to both eyes) the volunteer lost the ability to judge distance. More recently, a pilot who had been applying TEPP and was having great difficulty in landing his plane found that his vision cleared when he closed one eye, and he landed without further difficulty (90).

The expression "chronic disease" is properly reserved for sickness which persists or recurs with the result that scarring, atrophy, or other tissue injury, which the pathologist recognizes as evidence of a prolonged injury, occurs. Among the pesticides, compounds of lead, arsenic, and mercury long have been recognized as potential causes of chronic injury, although they also may produce acute damage. Many of the newer pesticides may produce illness after repeated exposure; this frequently is called chronic poisoning, but this usage misses an important distinction. To be sure, sufficient repeated dosage with any of the organic phosphorus insecticides will produce a depression of blood cholinesterase activity during exposure. However, if clinical poisoning does occur, it is acute, and recovery of blood cholinesterase or physical well-being is just as fast as in persons whose blood cholinesterase has been reduced to the same degree by a single dose. The full picture of chronic poisoning has not been demonstrated for any of the new pesticides (unless one considers the organic mercury compounds as new). However, the recurrence of convulsions has been observed in men and rats months after dermal exposure to dieldrin was discontinued. This indicates a certain chronicity, although the mechanism remains unknown (63).

Considerable attention has been paid to the potentiation between certain pairs of organic phosphorus compounds (27, 34, 46, 47, 82, 83, 84). DuBois (34) has shown that EPN (*O*-ethyl *O*-*p*-nitrophenyl phenylphosphonothioate) blocks the enzymes which normally convert malathion (*O,O*-dimethyl dithiophosphate of diethyl mercaptosuccinate) to relatively harmless materials; the conversion of malathion to the toxic malaoxon is not blocked and thus the body's defense against malathion is crippled. It is supposed that potentiation between other pairs of organic phosphorus compounds depends on a similar mechanism. A case of human poisoning has not been reported in which it seems necessary to invoke potentiation in order to explain the dosage-response relationship, but the possibility of potentiation certainly has to be kept in mind in connection with occupational exposure.

ROUTES OF EXPOSURE

It may be nearly as valuable to know the magnitude of exposure by different routes as to know the toxicity of a compound. It has been established for some time, on the basis of animal experiments, that many of the newer pesticides, except DDT, are absorbed easily through the skin even when not in solution. This property has been associated correctly with cases of poisoning following massive skin exposure, but the importance of dermal absorption in the more ordinary cases of occupational illness frequently has been missed. This failure of association was caused partly by a natural tendency to think in terms of the older economic poisons, most of which offer a serious threat by the oral route only, or in terms of the dusts, fumes, and gases, which are seriously dangerous by the respiratory route only and which constitute major industrial hazards. Another reason for past failure to associate dermal exposure with clinical effects was a lack of appreciation of the magni-

tude of dermal exposure which spraymen and mixers commonly receive. It has been shown for parathion (9), malathion and chlorthion (29), DDT (110), and dinitro ortho-cresol (10) that the "imperceptible" dermal exposure under practical conditions of agricultural or residual spraying is from 2 to 494 times as great as the respiratory exposure, even when only the face, neck, and lower arms are exposed. If clothing is less complete, the dermal exposure will be even greater. The fine mist which spraymen encounter is often imperceptible as it falls, but in a short time it leaves an easily visible deposit on eye glasses, wrist watches, or any other impervious, polished objects worn on the face or arms. This so-called imperceptible mist is what was measured in the research cited above, and it is in addition to spillage or any other gross contamination. Because DNOC (4,6-dinitro-ortho-cresol) is a brilliant yellow dye, it was possible for Edson (39) to compare the total skin contamination (whether from spillage or drift) with the blood level; he found a striking and consistent relationship both in factory workers and in spray operators. In one man, a single contamination caused a rise of blood DNOC from 6 $\mu\text{g.}$ per ml. to 30 $\mu\text{g.}$ per ml. during the next 24 hr. When 1 gm. of DNOC, as a slurry inside a rubber glove, was kept in contact with a human subject's hand for 8 hr. the blood DNOC level rose to 15 $\mu\text{g.}$ per ml. within 24 hr. This concentration of DNOC would indicate the presence of just over one-tenth of the total applied dose in the blood.

Technical questions remain about the relationship between the degree and timing of skin exposure, the rate of absorption, and the appearance of enzyme changes or clinical illness. However, there is no doubt that the measured dermal exposure of spraymen is adequate to account for the majority of cases of occupational poisoning by many newer pesticides. On the contrary, with exceptions mentioned below, measured respiratory exposure is entirely inadequate to explain the results. Oral exposure to pesticides during ordinary spraying has not been measured, but the amount ingested in various ways, such as eating with unwashed hands, appears to be small in most instances.

The protective effect of ordinary clothing and of special devices has been measured in two studies of DDT spraying (62, 110). The clothing factor is implicit in a comparison of the results of Wolfe *et al.* (110) on men with customary exposure and the results of Fletcher *et al.* (44) on men with exceptionally complete protection. The protective effect of clothing—and thus, the importance of skin exposure—is shown by the inverse relationship between exposure and the occurrence of symptoms in men applying dieldrin indoors (63).

Workers among crops previously treated with parathion may be poisoned by the residues. An episode was reported first by Ingram (66), but recently the matter has been studied more extensively by Quinby & Lemmon (89) who reported 12 episodes involving at least 149 persons of whom 78 showed clinical poisoning. The cases were distinguished by their mildness and by the high incidence among the same groups. In most of the episodes, the residues

were relatively fresh, but in a few the residues were 12 to 33 days old. The mechanism by which some residues remained active for more than a week has yet to be explained.

Respiratory exposure may be important, especially if the exposure is in closed spaces and the compound has a high vapor pressure or is dispersed as a fine aerosol. The volatile fumigants present their hazard almost exclusively by the respiratory route. The danger of certain alkyl mercurial compounds by this route is great, though less obvious.

What has been said above about occupational poisoning is not meant to underrate the importance of oral exposure when it does occur. Most cases of poisoning in children involve ingestion.

SIGNIFICANCE OF INCIDENTAL EXPOSURE

By incidental or environmental exposure is meant any exposure which is neither voluntary nor strictly accidental. Thus, incidental to treating a crop, some spray may fall in neighboring fields or suburbs without any intent to treat those areas and without any accident in the usual sense.

There is evidence that the release of lindane vapor indoors by vaporizers leads to the development of urticaria or other manifestations of hypersensitivity in a few people (23, 24, 72). Overheated vaporizers have produced fumes which were highly irritating to everyone who encountered them even momentarily (23).

As mentioned above, there have been complaints about incidental exposure to sprays or dusts applied to crops. With rare exceptions, crop dusts are applied in such a way that they are only temporarily air-borne. The persistence of the material in the air depends on particle size, particle density, and height of release. Dusts tend to remain suspended longer than sprays. Thus, it happens that one sometimes can see a relatively discrete cloud of dust carried for a quarter of a mile or more from the point at which it was released by an airplane. However, good agricultural practice requires that application be made when conditions (including lack of wind) permit the pesticide to settle on the crop for which it is intended.

In a study by Batchelor & Walker (9), the highest concentration of parathion found inside an orchard during spraying and dusting operations was 0.74 mg. per m.³. Some investigators (18, 91, 97) have found similar or lower maximal values. The values obtained by Kay *et al.* (69) for spray and by Braid *et al.* (17) for dust were significantly higher, ranging as high as 15.0 mg. per m.³. High concentrations are favored by wind or by the use of dust. Loading and mixing operations generally involve higher atmospheric concentrations than spraying and dusting operations under the same weather conditions (18, 19). Thus, Batchelor & Walker (9) found as much as 5.53 mg. per m.³ during loading and mixing. Windy conditions may largely eliminate the difference (69). Several studies have shown that the concentration of parathion in the air over treated fields falls to negligible levels in a short time (18, 69, 91, 97).

The highest concentration detected at a place which might involve fairly extended, nonoccupational exposure was 0.02 mg. per m.³ (9). Braid *et al.* (17) showed that persons 50 feet directly downwind from a dusting operation may receive a few minutes of exposure, at a rate as high as 3.6 mg. per m.³, as the dust cloud drifts over them. These and the other values given above for parathion may be compared with the maximal allowable concentration of 0.1 mg. per m.³ per 8 hr. of exposure to the compound as established by the American Conference of Governmental Industrial Hygienists (5). Emphasis has been put on parathion because it is the most toxic economic poison used so extensively and also because it has been most studied.

Measurement has shown, as one would expect, that the atmospheric concentration of parathion (17) and other insecticides (29) decreases approximately exponentially when the insecticide is carried downwind from a point source. However, Culver and his associates (29, 30) have pointed out that a cloud of insecticide tends to spread out as it drifts so that an observer standing in the drift at a distance from a moving sprayer is exposed for a longer time than a person standing nearer the spray machine. The longer time spent in the dilute cloud may produce almost as much exposure as a shorter time in a more concentrated cloud. In any event, if a cloud of insecticide does drift, it exposes a given area only briefly. People who might otherwise receive unintentional exposure get considerable protection from the direct application of crop poisons by aircraft if they are indoors even with the doors and windows open (21, 30).

Thus, the exposure of persons who happen to be near treated fields is small and transient. By contrast, the exposure of men who actually apply pesticides is far greater in intensity and especially in duration. The surveys mentioned above indicate that crop dusting, even with the most poisonous compounds commonly used, does not present a measurable hazard to persons who are exposed incidentally, although it may present a hazard to those who do the work.

Economic poisons are applied purposely, and they differ in this way from ordinary air pollutants, the release of which is more or less accidental and even may represent the loss of a useable by-product. Incidental exposure to crop dusts is generally momentary and thus different from the usual exposure to smog. Each crop dust is a clearly defined formulation of known toxicity, while smog is a variable mixture of compounds, only some of which have been evaluated or even identified. The most hazardous route of exposure of crop workers to pesticides is dermal; the important route of exposure of all of us to smog is respiratory.

There have been no cases of illness in this country from insecticide residues on food when formulations have been used according to directions. The same is true in Britain (39). However, there have been several instances in which insecticides used quite improperly on foods have led to poisoning. Nicotine sulfate residues caused illness in 11 persons who ate mustard greens which a farmer had sprayed with double the recommended dosage and then

sold the next day. Even two weeks after spraying, the residues on this particular crop were as high as 69 to 123 p.p.m.; the official tolerance is 2 p.p.m. (73). Two families were poisoned when toxaphene sprays were applied contrary to directions to collard greens and chard (75). The residue on the collards averaged 3315 p.p.m., and 3126 p.p.m. remained after the greens were washed three times. If toxaphene is applied to such crops at all, it is supposed to be used on seedlings only in such a way that no residue is present at harvest.

Related to the improper treatment of food with pesticides is the contamination of food during shipment. In one instance, the contamination of flour by endrin during shipment led to the poisoning of 59 people who ate bread made from the flour. Much of the contamination probably came from use of the flour sacks to cover the loaves as they rose (31).

A number of people have been genuinely concerned by articles which allege that insecticides, and especially residues of them on foods, are the cause of many human and animal diseases. DDT particularly has been incriminated. Papers by a very small number of physicians (12 to 16) have continued to receive sensational publicity in a certain segment of the popular press (33, 76 to 80, 92). DDT has been claimed to be the direct cause of a virus disease (later identified as influenza) (1, 2), a psychoneurotic syndrome, and a contributing cause of poliomyelitis, hepatitis, cardiovascular disease, and cancer, as well as of several diseases of animals. Less publicized claims have attempted to relate insecticides with fungus infection, allergy, sinusitis, gastrointestinal upsets, dehydration, alkalosis, malnutrition, pneumonitis, and insanity.

It is important to realize that the alarm which has been expressed is not shared by scientists who actually have studied insecticides nor by the vast majority of physicians. During years of investigation, it has been impossible to confirm the allegation that insecticides, when properly used, are the cause of any disease either of man or animals. When misused, however, they may produce poisoning.

Some of the diseases which are claimed to have increased because of DDT, in fact, have shown no increase. Some of the other diseases are increased largely because of the changing distribution of age groups in the population of the United States. Because of improvements in the control of communicable diseases of children and adults, a greater proportion of people live to be old enough to have cancer and those forms of heart trouble which strike in middle or old age. It is true that there are a few diseases, such as lung cancer, which have shown a real, as well as a relative, increase. However, as with cancer of the lung, the trend began before the newer insecticides were used and appears to be much better explained by other factors. Furthermore, animal studies indicate that DDT is not carcinogenic (11, 56).

In the clinical studies carried out by the Public Health Service, particular attention has been paid to workers who, because of their occupational exposure, came in contact with much more DDT than people in the general popu-

lation. It is a general principle that, when large samples are considered, poisoning can be expected to appear most quickly, frequently, diversely, and severely in those persons most extensively exposed. This is a principle which alarmists appear to have ignored.

EXPERIENCE IN OTHER COUNTRIES

As suggested above, the safety record of the newer pesticides in the United States is good. The same is true in Canada and England, yet the picture is far from universal. The greatest difficulty has involved parathion under a variety of trade names. In spite of the poor reporting of cases and the relatively restricted use of this and other compounds, the majority of fatal cases of parathion poisoning are known to have occurred in underdeveloped countries. Thus, the total number of deaths caused by parathion in the United States since the commercial introduction of this agricultural chemical in 1947 is less than 100. By contrast, 96 cases of parathion poisoning with 13 deaths were reported from a small portion of the cotton-raising section of Brazil in a single year. More than 100 fatal cases were reported in India in 1958 (68). In the same year there were 74 cases, including 67 deaths, from the Province of Syria, United Arab Republic, and 20 deaths occurred from the same cause in Jordan (112). For some reason, which is not at all clear, there has been an average of over 1500 cases of parathion poisoning with 336 deaths per year in Japan for several years (48, 64). In each parathion case, illness was acute and frequently followed a single massive exposure.

The situation with dieldrin is quite different. There are a few isolated cases in which drinking of a solution or suspension has led to poisoning. However, the vast majority of reported cases have involved spraymen who worked for weeks or months before becoming sick. Furthermore, the onset of illness did not follow an accident but just routine exposure. Cases from Venezuela, Ecuador, Nigeria, India, and Indonesia have been reviewed (59, 63), and cases from several other countries have been recognized but not reported in publication. At least 90 persons showed full epileptoid convulsions and 11 have shown recurrence of illness several months after last exposure to the compound. The occurrence of illness among the spraymen on different projects is proportional to the intensity and duration of occupational exposure. Based on measurements of exposure during indoor spraying with DDT (110) and on toxicity tests of dieldrin in animals, it is clear that the dermal exposure of spraymen is adequate to account for observed illness. Fletcher *et al.* (44) measured the dermal exposure of spraymen in a program in which no poisoning had occurred. These men used the best available protective equipment and observed good personal hygiene under strict supervision. Measured skin exposure to dieldrin averaged 1.8 mg. per kg. per day of which an unknown part was removed by bathing. This value may be compared with approximately 20 mg. per kg. per day calculated for the same concentration of spray and more nearly average protective measures (110).

PREVENTION OF INTOXICATION

We live in an age of responsibility as well as in an age of chemistry. The tremendous interest which exists in the prevention of intoxication depends on this sense of responsibility. Advances in the control of communicable disease afford an opportunity to attack other health problems, such as poisoning, which for some years have remained static or have shown only slight amelioration. Of course, responsibility in the use of chemicals has paralleled their introduction into industry and agriculture; if this had not been true, the safety record of chemicals would have deteriorated rapidly instead of improving slowly.

Measures for preventing injury by pesticides must be based on a firm knowledge of their physical, chemical, and biological properties. Once the research has been done, certain appropriate safety measures may be enforced under law while other measures and a proper attitude toward safety may be better communicated by education. It may be found that protective clothing, respirators, masks, air-conditioned cabs, special factory ventilation, or a wide variety of other devices may be applicable to a particular problem. In addition to regulations, education, and protective devices, a co-operative working relationship between those who apply pesticides and those who may receive incidental exposure as a result of the application will do much to promote the welfare of all in the community.

In the United States, economic poisons are subject to extensive state and federal regulations. In a few instances, specific poisons are subject to local regulations also. There are three main kinds of laws: (a) labeling laws, (b) laws governing residues on food, and (c) laws regulating use.

The federal labeling law is entitled "The Federal Insecticide, Fungicide, and Rodenticide Act of 1947" (100, 101, 104). Similar laws exist in most of the states, and these laws have been reprinted in a single collection (22). Labels offer an indirect control of use. Federal regulation of pesticide residues on foods is carried out under the "Federal Food, Drug, and Cosmetic Act" (103), as amended (106). Many states have similar laws.

The federal government does not regulate the use of a material directly since the use is always carried on locally. Only one state has a very extensive direct regulation of the use of pesticides: California requires that permission be obtained for each separate application of any of several of the more toxic compounds (19). However, this law is directed as much toward the protection of crops from the indiscriminate use of hormone-type weed killers as it is directed toward the protection of workers. Nowhere in this country are regulations for the protection of workers who apply economic poisons developed to the same high degree as that exemplified by comparable laws in the United Kingdom (54).

A number of states require the examination and licensing of commercial pest control operators or the special licensing of pilots who apply economic poisons, or both. In addition to the laws already mentioned, manufacturers

and packagers of pesticides may have to conform to laws regarding the transportation of dangerous materials (105) and laws regarding caustic poisons (102). Some local governments issue regulations to cover specific problems. For example, the City of Chicago and some other places have regulated the sale of thermal vaporizers for lindane.

Of the three kinds of regulations, the one concerned with labeling is by far the most important. This conclusion is indicated by a review of the safety record of pesticides in different countries. Thus, the United States and the United Kingdom have effective provisions for the labeling of pesticides and both have good safety records, although their regulations regarding residues and use are quite different. On the contrary, certain other countries with advanced technology—not to mention underdeveloped countries—have inadequate regulation of pesticide labels and a poor safety record. No matter whether labeling is enforced by law, as in the United States, or by less formal agreement between government and industry, as in England (39), good labeling is the most important single means of educating people in the proper procedures and precautions for using pesticides. Publications, posters, safety campaigns, and special courses are useful but not so universally applicable.

Before a label is approved under the federal law, research must be carried out and data presented which show that, if used according to directions, the pesticide will produce the biological result claimed and will be safe. Dangers are cited and precautions are clearly stated in the label before it is approved. Much research usually is necessary for label approval. Labels are written in terms of specific uses only, and, when a label is first issued, the poison may be restricted to a single use. Other uses may be permitted later if additional research justifies them in accordance with the above criteria. Good labeling also applies to the residue problem, because labels are not accepted for uses involving crops until residues have been determined, proper intervals from treatment to harvest have been set, and tolerance limits have been established.

The laws which regulate pesticides in the United States have been a great direct and indirect cause of research on the toxicology and pharmacology of these compounds. This research is a continuing process because of the new pesticides which are introduced and the new uses which are developed for existing compounds. However, in addition to this research which is required in accordance with the laws mentioned above, there is a need for research on the effect of pesticides on persons who use them in public health or agriculture or who encounter them in their environment as residues in food. Studies of this kind carried out by the Public Health Service offer a further protection to agriculturists and to the general population against injury by the ever increasing number and variety of economic poisons.

DIAGNOSIS AND TREATMENT

Information on the diagnosis and treatment of poisoning by pesticides is becoming more readily available. Useful recent books are those by Sollmann

(96), von Oettingen (107), and Gleason *et al.* (51). A more specialized work is the *Clinical Memoranda on Economic Poisons* (25).

A discussion of the diagnosis and treatment of poisoning by individual compounds is beyond the scope of this review. However, it should be recalled that cases of human poisoning by the following pesticides are on record: inorganic compounds (lead arsenate, arsenic trioxide, Paris green, sodium cyanide, thallium, sodium fluoride, cryolite, mercuric chloride, sodium chlorate, barium carbonate); other metallic compounds (several organic mercury compounds); botanical insecticides (pyrethrum, rotenone, nicotine); chlorinated hydrocarbon compounds (DDT, TDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane], BHC [1,2,3,4,5,6-hexachlorocyclohexane], chlordane, aldrin, dieldrin, endrin, toxaphene); fungicides (pentachlorophenol, ziram); organic phosphorus insecticides (parathion, methyl parathion, tetraethyl pyrophosphate, demeton, Diazinon, Phosdrin, mipafox, schradan, Dipterex [dimethyl(2,2,2-trichloro-1-hydroxyethyl)phosphate], DDVP [2,2-dichlorovinyl dimethyl phosphate], malathion, Thimet, Trithion [S-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate]); nitrophenyl compounds (dinitro-ortho-cresol); hormone-type herbicides [2,4-D(dichlorophenoxyacetic acid)]; rodenticides [strychnine sulfate, ANTU (alphanaphthylthiourea), sodium monofluoroacetate]; fumigants (cyanide, methyl bromide, dibromoethane); and miscellaneous pesticides (lethane, naphthalene).

A new development of great interest is the use of 2-pyridinealdoxime methiodide (2-PAM), diacetyl monoxime (DAM), and related compounds for the treatment of poisoning by organic phosphorus compounds. Both 2-PAM (55, 85) and DAM (55) have received clinical trial with somewhat encouraging results. The compounds are not yet generally available, but they deserve much further study. They act by releasing cholinesterase from what would otherwise become an irreversible complex with organic phosphorus compounds. This action is entirely different from that of atropine, and the two treatments supplement one another.

A development of a different kind, which may prove useful in more cases of poisoning, is the measurement of effectiveness of removing poisons that have been swallowed. In a study of sodium salicylate, it was found that vomiting induced immediately or even 1.5 hr. after ingestion was more effective than gastric lavage in removing the poison (4). Several factors, including the fact that some people cannot be induced to vomit, indicate that gastric lavage must be used at times. However, more emphasis should be put on induced vomiting in the treatment of poisoning by mouth. No doubt there may other measures used in the treatment of poisoning which ought to be re-examined objectively.

Poison control centers have become very popular. The first was established in 1953, and there are now some 260 operating in 42 states. The centers enjoy a variety of services from the National Clearing House for Poison Control Centers, including statistical assistance and the publication of comprehensive progress reports (20). The contribution of the centers in main-

taining records of antidotes and of the ingredients of trade-name products is well recognized, so much so that one might suppose that physicians had never treated poisoning before. Probably much more important is the safety education which some of the centers have money and staff enough to carry out (3, 67). This education, carried out best by visiting nurses of the health department, promises to be very important in accelerating the improvement in the safety record of poisons.

CONCLUSION

The safety record of the newer compounds is good in the United States and several other technically advanced countries largely because of careful labeling. In countries where labeling is poor or where illiteracy or irresponsibility tend to vitiate labeling, hundreds of cases of human poisoning have occurred. Because of the usefulness of economic poisons in the control of vector-borne diseases and a wide range of agricultural pests, it should be our purpose to improve the safety of all pesticides, both old and new, in all countries, in order that their benefits may not be compromised. Since many new pesticidal compounds and some new uses have been introduced in the last 10 years, it is expected that many problems will require continued surveillance. The very existence of highly active compounds, some of which are stored in the tissues of a majority of people, requires prolonged investigation of those whose occupations enforce exposure many times greater than that of the general population. However, there is nothing to be gained in the long run by irresponsible statements that nothing now is known of the toxicology of the newer pesticides, or that no legal control of their use exists, or—in the absence of epidemiologic proof—that a wide variety of illnesses from which mankind has suffered for generations is now caused by intoxication by the newer economic poisons.

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BIOLOGICAL RELATIONSHIPS BETWEEN LICE (ANOPLURA) AND MICROBIAL AGENTS¹

By F. WEYER

Bernhard-Nocht-Institut für Schiffs- und Tropenkrankheiten, Hamburg, Germany

INTRODUCTION

Normally the bodies of sucking lice (Anoplura) are free from pathogenic microorganisms. Sterility of the intestinal contents is one of the important factors determining the optimal conditions for life of lice, which can even rid themselves of microbial agents—at least to a certain degree. Symbiotes are the only permanent and obligatory residents of the bodies of lice. They furnish nutritive substances which are indispensable for the growth and reproduction of their hosts [Aschner (3); Puchta (61)].

On the other hand, lice are known to be vectors of spirochetes and rickettsiae; epidemic typhus, trench fever, and relapsing fever are "louse-borne diseases." Not until relapsing fever and certain rickettsial diseases were investigated, were lice and their role as transmitters of disease agents studied more thoroughly [Buxton (8); Hase (28)]. Today we have a fair knowledge of the relationships between the sucking lice and some of the more important pathogenic agents. We know hardly anything, however, concerning those internal biochemical processes which govern the association of lice and microbial agents. For practical reasons, the main object of investigation has been the body louse, *Pediculus humanus humanus* Linnaeus. Our knowledge regarding the same relations between animal lice and microbial agents are much more incomplete.

While fundamental observations on the role of lice as vectors of diseases were made years ago, recent investigations have broadened our knowledge. Spirochetes and rickettsiae are probably the only true parasites of sucking lice. Hence we will deal mainly with these parasites in this paper; only the more significant observations and experiments that pertain to the behavior of other blood parasites borne by lice, and their transmission, will be reported. Here the relationships between host and parasite are either accidental and short-termed or have not been sufficiently explained as yet. Since many of the observations made are based upon laboratory experiments, the techniques that have been applied have a bearing on the results and must, therefore, be briefly discussed.

NOTES ON RESEARCH METHODS

For experimental work, the rearing of body lice under laboratory conditions is essential. For detailed descriptions of breeding methods see Nauck & Weyer (50); Reichenow, Vogel & Weyer (63); Sikora (67); and Smith & Eddy (69). True head lice, *Pediculus humanus capitis* DeGeer, and crab lice,

¹ The survey of the literature pertaining to this review was concluded in April, 1959.

Phthirus pubis (Linnaeus), can experimentally be maintained on man for a limited time only. Animal lice are reared on their natural hosts. *Haematopinus suis* (Linnaeus), normally living on pigs, will survive for a few days if fed on human blood [Weyer (83)]. The lice are kept in wooden or metal cages, one side of which consists of gauze to permit sucking, and under as sterile conditions as feasible. Normal reproduction—yielding an excessive number of lice—can be achieved by maintaining the lice at 30°C., and feeding them for 30 min. daily. Other methods were developed in order to avoid the somewhat difficult procedure of feeding lice on man; for example, animal or artificial membranes were used, the nutriment being defibrinated blood [Fuller, Murray & Snyder (23); Haddon (27)]. Here, however, lice can be maintained only for a limited time while their propagation rate is low.

A considerable improvement of rearing methods was achieved when strains of body lice could be experimentally selected so as to develop normally if fed on rabbits [Culpepper (16); Pshenichnov & Noskova (60)]. Contamination of the colony by bacterial infections, however, occurred more frequently than after feeding the lice on man. Feeding on man will also considerably facilitate simultaneous experimental work with a large number of infected lice.

To investigate the relationships between lice and the microbial agents they harbor, lice gathered from sick persons or animals under natural conditions can be directly or indirectly examined for microorganisms, although better information will usually be obtained by experimental inoculation of lice. Normally lice take up microorganisms only when sucking; invasion of the stomach via the rectum—proceeding from moist feces, for example—will occur only if the individual louse is old or weakened by sickness. Lice can be inoculated in the natural way by feeding them on infected hosts. Body lice do not necessarily require human blood; for a single time, at least, they may be fed on monkeys, guinea pigs, rats, mice, or rabbits. Mortality resulting from feeding lice on a nonspecific host can be decreased by holding the lice at 37°C. for 1 or 2 hr. immediately after feeding and offering them human blood soon after [Cabasso (9)]. For experiments with strains adapted to rabbits, the lice may be fed on rabbits that have previously received an intravenous injection. As proved by Snyder & Wheeler (70), rickettsiae will remain in the blood of rabbits—which were inoculated by use of a yolk-sac suspension, for instance—for at least three days. Small amounts of microbial agents may be intradermally injected (bleb technique), thus permitting lice to take up the agents when sucking from the bleb area. This method is particularly useful for infecting the youngest larval stages of lice. Inoculation is greatly facilitated if the lice are fed through membranes, since a larger amount and a known concentration of microbial agents may be added to the blood used as nutriment.

The study of the relations existing between lice and microbial agents was considerably advanced by the discovery that experimental inoculation of lice by means of very thin glass capillaries is possible [Weigl (79)]. This method

has proved useful until today, as it is both simple and exact [Kryński & Radkowiak (34); Weyer (86)]. By application of this technique, not only blood but also suspensions of organs and concentrated doses of agents may be inoculated into the stomach of the louse. The behavior of the microorganisms and the reactions of the louse will be the same if the agents invade the louse via the rectum or in the natural way, i.e., via the esophagus. Furthermore, lice may be intracoelomically inoculated [Weyer (86)]; for this female specimens are suited best, because inoculation can be made via their genital openings. Both inoculation methods are used not only for research work but also for maintenance of pathogenic strains in lice.

NONSPECIFIC OR INSUFFICIENTLY EXPLAINED RELATIONSHIPS BETWEEN LICE AND MICROBIAL AGENTS

Lice, which are stationary blood-sucking ectoparasites, have frequently been suspected of transmitting other microbial agents than spirochetes and rickettsiae because their infestation of man is often accompanied by various other diseases. Hence, some observations and experiments will be discussed which were devised to demonstrate whether certain agents will survive and propagate in lice and whether lice must be considered transmitters of these agents.

Suspected or tested microorganisms include viruses, bartonellae, bacteria, protozoa, and filariae. Negative results were obtained by experiments with filariae; certain species of *Plasmodium*, *Leishmania*, *Anaplasma*, and *Leptospira*; *Trypanosoma evansi* (Steel); *Toxoplasma gondii* Nicolle & Manceaux, and the agent of the swamp fever. The number and methods of the experiments concerned, however, do not always permit final conclusions. Besides body lice, various animal lice were investigated. It is worth mentioning that *T. gondii*—injected intracoelomically—not only lived up to 13 days in the hemolymph of the louse, but also remained virulent and capable of reproduction, though no multiplication took place in the hemolymph itself [Weyer (82)]. Positive results were gained from experiments with the viruses causing trachoma [Cuénod, Nataf & Loukitch (15)], lymphocytic choriomeningitis [Milzer (40)], rabbit myxomatosis [Button (7)], and yellow fever [Philip (57)]. But all these experiments proved only that these agents may be ingested by lice when sucking and that they will survive for a certain time in the body of the arthropod.

We are better acquainted with the relationships existing between lice and certain bacteria. Alverdes & Bieling (2) administered various bacteria to body lice either by feeding the latter on rabbits or inoculating them via the rectum. Both methods yielded the same results: certain large gram-positive cocci, which are occasionally found on or in the body of lice, remained in the stomach lumen of lice for a longer time—their reproduction rate being low—without harming their hosts. Influenza bacilli, pneumococci of Type I, and *Bacillus cereus* var. *mycoides* (Flügge) could no longer be demonstrated after a maximal period of 48 hr. On the other hand, a true infection was gained

after the lice had been inoculated with typhoid, paratyphoid, and colon bacilli. The bacteria invaded the stomach cells and multiplied intensively. The infection caused a rapid destruction of the stomach cells, which had become distended, and led to a detachment of the mucous membrane from which the lice died. The bacteria were voided with the feces.

Similar experiments were carried out by Milner, Jellison & Smith (39). The lice ingested blood containing bacteria either through membranes or by feeding on infected rabbits. Principal investigations were made on *Salmonella enteritidis* (Gaertner); parallel tests were carried out on *Salmonella typhimurium* (Loeffler), *Salmonella pullorum* (Rettger), *Salmonella paratyphi A* (Kayser) and *Shigella dysenteriae* (Shiga). *S. enteritidis* multiplied in lice rapidly, the maximum number being reached after 6 to 8 hr. Ninety per cent of the lice died on account of the infection within 48 hr. *Salmonella* survived in dead lice and feces of lice for more than 1 yr. and could be transmitted to rabbits by means of suspensions of triturated lice and feces. Kryński, Kuchta & Becla (33) transferred suspensions of *Proteus* cultures (OX 19) to lice by Weigl's method. The majority of lice died within 48 hr. as a result of degeneration of the mucous membrane of the stomach caused by a toxic effect. Only a few lice succeeded in overcoming the infection. The bacteria remained in the lumen of the stomach and did not attack the stomach cells.

Lice have also been suspected as carriers of leprosy and plague bacilli, although investigations of this matter are rare and incomplete. In several cases acid-fast bacilli were found in lice infesting people who suffered from leprosy, but it still remains to be proved in some cases whether these acid-fast bacilli were really leprosy bacilli. Marchoux & Chorine (37) were able to transfer rat leprosy by inoculation of healthy rats with suspensions of lice collected from infected rats. By the same method plague bacilli could be transmitted to rodents by body and head lice [Long (36); de Raadt (62); Swellengrebel & Otten (76)]. In several instances, plague bacilli could be demonstrated in various rodent lice [Link (35) and others]. Observations by Blanc & Baltazard (5), who claim that pig lice are capable of transmitting plague bacilli from infected to healthy guinea pigs by biting, have not been confirmed. The same authors found that lice harboring plague organisms acquired this infection from humans during the septicemia, and that the agent may remain infective for at least 7 days in lice and 9 days in the feces of lice.

In several cases, transmission of tularemia to mice, guinea pigs, and rabbits by various rodent lice was achieved [Francis & Lake (19); Girard (25); Parker (56) and others]. These findings were experimentally supported by Price (58, 59). The lice ingested rabbit blood which contained *Pasteurella tularensis* (McCoy & Chapin) through a membrane. Afterward the lice and their feces were preserved at various temperatures and relative humidities to test the longevity of the bacteria, which was mainly affected by the temperature. The bacteria remained alive in the feces for a shorter span than in the lice if humidity was very high, for a longer time than in lice if humidity was low. At 29°C. and a relative humidity of 0 per cent, for example, the bacteria survived for only 0.5 days on blotting paper, for 17.0 days in dead lice, for

11.5 days in fasting lice, while they were still alive after 22.5 days in louse feces, after 26 days at 20°C., and even up to 53 days at 4°C. Thus feces of lice proved to be a valuable medium for preservation of bacteria. Generally, all lice harbored bacteria after feeding on infected rabbits. The number of lice which continued to harbor bacteria was dependent on the amount of bacteria ingested. The number of lice containing no bacteria increased with the time elapsing since the infectious blood meal. In some lice, however, bacterial reproduction was high, bacteria of unchanged virulence being voided with the feces. Several lice harbored bacteria up to 35 days. Out of 13 healthy rabbits, on which infected lice were fed, 10 died of tularemia 5 to 16 days after the first feeding of infected lice.

There seem to exist certain relationships between lice and bartonellae. Cannon & McClelland (10), Crystal (14a, 14b), Eliot & Ford (18), Mayer (38), and Timmermann (77) were able to transmit *Haemobartonella muris* (Mayer) to rats using rat lice [*Polyplax spinulosa* (Burmeister)] as vectors. For this purpose, the lice were transferred from infected to healthy rats. Furthermore, Eliot (17) showed that *Eperythrozoon coccoides* Schilling of mice can be transmitted by *Polyplax serrata* (Burmeister). There had been no conclusive evidence of reproduction of these agents in lice. Crystal (14a, 14b), however, found that the infectiousness of the haemobartonellae is enhanced in lice within four to seven days after feeding on infected rats. In most experiments the mode of transmission had remained unknown. According to Crystal, transmission occurs normally by the rat crushing infected lice and inoculating itself by scratching and biting. Transmission may also occur directly by bartonellae remaining on its mouthparts if lice, after having fed on an infected rat, proceed immediately to feed on a second rat. Lice have also been suspected of transmitting *verruca peruviana* in Colombia [Weinman (81)]. This assumption could not be proved by experiments with *Bartonella bacilliformis* (Strong *et al.*) [Wigand & Weyer (93)]. In the course of these experiments, body lice were inoculated either rectally or in the natural way by feeding them on infected monkeys. The bartonellae were never seen to establish themselves or multiply in lice. It is worth mentioning, however, that the number of bartonellae greatly increased in the hemolymph of lice, if they had been intracoelomically inoculated, and could later be cultivated on culture media.

Most results of the investigations previously mentioned indicate only that microbial agents, present in the blood of mammals, will be ingested by lice, where they may remain virulent for some time in the stomach, and may be—at least to some extent—voided with the feces. There is nothing peculiar about this, and it is quite natural, too, that such lice may become a source of infection, either by being crushed on the skin or by means of their feces. Here lice must be regarded as only mechanical transmitters, similar to other blood-sucking arthropods or house flies, though of little practical importance because of their stationary mode of life. There is no evidence of intimate biological relationships between these blood parasites and lice.

Differences in reaction of lice to infection by these blood parasites may

arise primarily from properties of the agents themselves. Some of these will die in the stomach, while the majority will probably be voided with the feces. Frequent blood meals and active digestion cause regular rinsings and cleanings of the alimentary canal, which may be effective, even in cases of severe infections, after several days, as is the case with *P. tularensis*. Some agents, however, particularly if present in great numbers, are capable of remaining alive in lice and multiplying either extracellularly in the lumen of the stomach or intracellularly in the cells of the stomach wall. The reasons for this difference in behavior are not known. In some cases, e.g. with *Proteus* and *Salmonella*, growth of the agents will be attended by a severe toxic effect. Even if the invasion of the agent must be considered a true infection, the quick lethal effect on lice makes a biological relationship appear doubtful. Since *P. tularensis* is capable of reproduction in the alimentary canal of lice and will be voided with the feces as a virulent agent even after weeks, it may be that this relationship represents the first stage of a biological adaptation between both organisms.

The practical consequences of these facts are probably insignificant. Infection of lice on man requires a severe septicemia, which will hardly be sufficient in cases of tularemia and salmonellosis. If louse infestation is extensive, lice—in association with epidemic typhus and relapsing fever—may play a role in spreading *Salmonella* [Milner, Jellison & Smith (39)]. Here, as in the case of tularemia, the feces containing the microbial agents must be considered the actual danger. There is, of course, no evidence of transmission by biting or by transovarial transfer [Price (59)].

RELATIONSHIPS BETWEEN LICE AND RICKETTSIAE

Body lice are the natural hosts and vectors for *Rickettsia prowazekii* da Rocha Lima, the agent causing epidemic typhus, and for *Rickettsia quintana* Schmincke, causing trench fever. Larval and adult stages are equally susceptible to the rickettsiae. After lice had been suspected of transmitting classic epidemic typhus rickettsiae for many years, the agent and its behavior in lice were described in detail for the first time by da Rocha Lima (65). Munk & da Rocha Lima (48) reported also on the role of lice as transmitters of *R. quintana* and on the behavior of this agent in lice.

R. quintana occupies an exceptional position among the rickettsiae which are pathogenic for man [Mooser & Weyer (45); Weyer (87)]. These rickettsiae do not invade the insect's stomach cells. Their reproduction is exclusively extracellular, either on the epithelial cells, where they frequently form a rodlike fringe, or in the lumen of the stomach. Viability and longevity of the lice are not affected, even if the rickettsiae grow rapidly. Once infected, lice will harbor the agents for life. As the rickettsiae remain confined to the stomach and will leave their hosts only by means of the feces, lice feces are the only source of infection for man. The assumption that, besides *R. quintana*, other rickettsiae less pathogenic or apathogenic for man will grow in lice extracellularly [Herzig (32); Mosing (47)] cannot be maintained any longer. We have good reason to believe that *Rickettsia pediculi* Munk & da Rocha

Lima and *Rickettsia weigli* Mosing are identical with *R. quintana* [Codeleoncini (14); Weyer (87)].

Another species of rickettsiae has been observed several times in lice which had been reared under laboratory conditions. This species, whose origin could not be detected, was named *Rickettsia rochalimae* Weigl [Sparrow (71); Weigl (80)]. The striking features of this species, according to the available reports, are its ability to multiply in the louse stomach both extra- and intracellularly without severely harming its host and its high infectiousness for lice. These rickettsiae, which are very resistant, will even invade healthy lice after short contact with the dried feces. Thus the occurrence of this species in a louse colony will lead to an infection of all lice within a few days. At present, laboratory strains of this species do not exist.

Although lice are considered natural vectors for *R. prowazekii* only, *Rickettsia typhi* (Wolbach and Todd)², the agent causing murine typhus, behaves principally in the same way as *R. prowazekii* when harbored by lice. Both species grow intracellularly. The rickettsiae which have reached the stomach invade the cells of the mucous membrane, where they propagate so intensively during a few days that the entire cell plasma will be filled with rickettsiae. Accordingly the cells of the mucous membrane will become distended until they cannot resist the pressure any more and will burst, shedding their contents into the lumen of the alimentary canal. From here the rickettsiae will be carried away by the feces, and then can invade humans via skin injuries or through mucous membranes. Most lice die 8 to 12 days following ingestion of the rickettsiae. This is a distinctive property of lice as compared to fleas, whose longevity is not affected by the rickettsial infection. In lice, the rickettsiae do not invade salivary glands or eggs. Under natural conditions they will reach the stomach only when lice suck blood; an infection of lice by contact with feces which contain rickettsiae does not take place.

Using the membrane technique [Fuller, Murray & Snyder (23)], Fuller (20) made a comparative study on the susceptibility of lice and cotton rats for detecting very small amounts of rickettsiae. Both methods proved to be of equal value. Furthermore, he fed lice through membranes on suspensions which contained rickettsiae and after this on rabbits [Fuller (21)]. Applying this method, he was able to maintain a strain of *R. typhi* through 19 passages for 95 days continuously in lice, and a strain of *R. prowazekii* through 5 passages for 79 days. *R. typhi* also multiplied if the lice were fed on human serum. The effect of a *R. prowazekii* infection on the longevity of lice was studied by Fuller (22), too. On an average the infected lice remained alive for 15.4 days at 25.6°C. (control lice 26.3 days), for 7.4 days at 31.9°C. (control lice 12.5 days), for only 2.4 days at 35.8°C. (control lice 2.4 days also). The dose of the

² Although the author prefers the name *Rickettsia mooseri* Monteiro for the agent causing murine typhus, the Editors have requested that the nomenclature as used in *Bergey's Manual of Determinative Bacteriology* be followed for members of the family Rickettsiaceae Pinkerton.

infectious material, which was increased to one thousand times the original amount, however, affected the longevity of the lice only slightly.

Pathogenicity of rickettsiae for lice arises from the fact that the stomach epithelium loses its functional properties. The main damage is done by a mechanical effect: the cytoplasm—the nucleus is never invaded—is consumed and destroyed by the intensively growing rickettsiae. The stomach cells may also be harmed, in cases of slight rickettsiae infections, by a toxic effect. This implies that pathogenicity is not a simple function of the number of microbial agents. The result is liquefaction and vacuolization of the cytoplasm at its basis and, in extreme cases, detachment of single cells or larger cell shreds. The destroyed cells cannot be replaced, since lice have no regeneration crypts like other insects. Hence the stomach epithelium of lice will be more or less injured if they suffer from a rickettsial infection. This state is externally recognizable by the reddish color of the lice, resulting from hemoglobin that was ingested when feeding and which now enters the hemolymph through the damaged stomach walls. Such "red lice" will survive for a few hours only. During this period rickettsiae may leave the stomach cells and invade the hemolymph, where they may continue to grow.

The normal development of rickettsiae in lice shows variations that are attributed partly to certain properties of the strains, partly to periodical fluctuations of virulence in the same strain. These variations become most evident when the growth rate of rickettsiae and their pathogenicity for lice are studied. Also, because of these variations, the first rickettsiae may be found in smear preparations of the stomach on the second day or not before the eighth day after inoculation, and the infection may prove lethal as early as the second day, or not at all. There are also certain properties of the lice which have a bearing on the infection, regulating it to some extent [Weyer (86)]. Although the same doses of infectious material were used, rickettsiae were rapidly growing in some lice of a batch, while they multiplied more slowly in others. There were also lice which appeared to be healthy, their stomach cells being only sporadically infected. Sometimes lice, whose entire mucous membrane had been invaded by the microbial agents, had a normal life span. Other experimental results can be explained only by suggesting that in some lice rickettsial growth is not only temporarily or permanently retarded, but that rickettsiae which have been multiplying after invasion of the cells are destroyed. Some observations [Reichmuth (64)] seem to imply that resistance to rickettsiae is related to certain geographical races which may occur within one population, such as in dark-pigmented lice. However, the evidence which is available cannot be considered conclusive as yet.

The number of lice which will become positive after feeding on a patient suffering from epidemic typhus depends on the extent of rickettsaemia. Usually this is sufficient for infection only during the first two weeks of illness, but lice may also become infected while feeding on people suffering from Brill-Zinsser disease, in which case rickettsaemia is low [Murray & Snyder (49); Weyer & Hornbostel (91)]. With trench fever, in contrast to the classic epidemic typhus, a rickettsaemia lasting for several weeks or even months—and sufficiently severe to allow infection of lice—is the rule.

Besides *R. quintana*, *R. prowazekii*, and *R. typhi*, there are also other species of rickettsiae capable of reproduction in lice. Species or strains that have been successfully transferred to the mucous membrane of the louse stomach, where multiplication took place, either by feeding lice on infected mice and guinea pigs or by rectal inoculation are: *Rickettsia rickettsii* (Wolbach) (strain Michoacan from Mexico, strain Bitterroot from U.S.A., one strain from Brazil); *Rickettsia conorii* Brumpt (one strain from North Africa, one strain from Kenya, one strain from South Africa); two strains of Siberian tick-bite fever (*Derma-centroxenus sibiricus* Zdrodovskiy); *Rickettsia akari* Huebner et al. (strain Kaplan from North America, one strain from Russia); *Rickettsia australis* Philip and one strain of *Coxiella burnetii* (Derrick) [Weyer (85, 86, 88, 89)]. Virulent rickettsiae were voided with the feces of the lice. Their number, however, was rather small. Sometimes—at late stages of the infection—the hemolymph, too, was invaded and there the rickettsiae continued to multiply. *C. burnetii* was capable of infecting other organs from the hemolymph. The strains could be easily maintained through passages in lice by use of suspensions of infected stomachs as an inoculum; without that the strains would change their properties. In several cases, maintenance of these strains proved to be easier than rearing *R. prowazekii*.

Regarding their pathogenicity for lice, the species and strains which were tested showed significant differences. Apparently pathogenicity was caused by a toxic effect. For their growth the rickettsiae preferred the protoplasm between the base of the cell and the nucleus. Even if only few rickettsiae were present, the cell base showed vacuoles and other symptoms of degeneration, in the course of which shreds of cells or the entire mucous membrane became detached. This proved to be lethal for the lice. Invasion of the nuclei was never observed, not even with rickettsiae capable of propagating intranuclearly in ticks or in tissue cultures. Strains of South African, Kenyan, and Siberian tick-bite fever, furthermore strains of *R. akari* and *R. australis*, showed the lowest pathogenicity. Pathogenicity was highest with a strain of *R. rickettsii* from Mexico and a strain of *R. conorii* from North Africa. It has not been established whether the differences between the strains of *R. conorii* and *R. rickettsii* which were tested must be considered strain- or species-specific.

Unexpectedly, corresponding experiments on the transmission of *Rickettsia tsutsugamushi* (Hayashi) to lice failed [Weyer (88)]. The following five strains were tested: Karp (New Guinea), Gilliam (Burma), Korea, Kato and Hokkaido (Japan), and one of *R. tamiyai* (Japan). Hence, this failure of transmission must be considered a species-specific property. The rickettsiae were not capable of invading the stomach cells. Evidently they were destroyed within a few hours after transmission or were voided with the feces.

The behavior of rickettsiae after intracoelomal transmission proved to be rather uniform. All strains tested, including the strains of *R. tsutsugamushi* as well as *R. quintana*, readily propagated in the hemolymph of lice and could be maintained through passages in lice for a considerable time by means of intracoelomic inoculation. Some species, e.g., *R. rickettsii*, grew better in the hemolymph than in the stomach cells. The fact that the rickettsiae multi-

plied mainly extracellularly is of biological importance. Only a few of them grew in hemocytes. In some instances (*R. prowazekii*, *R. quintana*, *R. conorii*) invasion of the stomach cells from the hemolymph was occasionally found. Sometimes *C. burnetii* grew also in the cells of the hypodermis and the fat body.

R. prowazekii, *R. typhi*, and *R. quintana* are capable of multiplying in crab lice as well as in body and head lice [Weyer (84)]. The blood-sucking animal lice are probably also susceptible to *R. typhi* and *R. prowazekii* [Weyer (90)]. The occurrence of *R. typhi* in rat lice (*Polyplax spinouloso*), where the rickettsiae grow in the same manner as in body lice, has been demonstrated several times [Mooser, Castañeda & Zinsser (44)]. The susceptibility of monkey lice [*Pediculus longiceps* Piaget and *P. albidus* (Rudow)] to *R. prowazekii* has been known for a long time [Blanc & Woodward (6)]. Weyer (83) inoculated pig lice (*Haematopinus suis*) rectally and intracoelomally with *R. prowazekii*. Invasion of the stomach cells followed exactly the same pattern as with body lice. The infection proved to be lethal for the lice. The rickettsiae multiplied readily in the hemolymph after intracoelomal inoculation, too. On the other hand, all experiments designed to render it possible for *R. quintana* to grow in the stomach cells or hemolymph failed. This agent seems to be a specific parasite of human lice.

The fact that the other rickettsiae, which are pathogenic for man, will grow in the stomach or in the hemolymph of lice, too, is of hardly any practical importance. Experimentally lice became infected by feeding on mice or guinea pigs. However, we do not know whether the rickettsiaemia of humans—suffering from rickettsialpox or tick-bite fever, for instance—will be sufficient to allow infection of lice, all the more since the amount of rickettsiae which are voided by infected lice with their feces is rather low as compared to an infection with *R. prowazekii*, *R. typhi*, or *R. quintana*. Demonstration of rickettsiae in lice under natural conditions, e.g., *C. burnetii* [Giroud & Jadin (26)] and *R. rickettsii* [Sylvia-Goytia & Elizondo (68)], must be considered accidental. If rickettsiae are capable of reproduction in lice, this does not necessarily prove that transmission under natural conditions is possible. Although *R. prowazekii* and *R. typhi* will readily grow both in lice and fleas, rat fleas are the significant vectors of murine typhus, while body lice are responsible for the spreading of the classic epidemic typhus. Lice may play a role in the transmission of murine typhus only if this disease occurs epidemically.

The fact that the stomach and the hemolymph of lice are suitable culture media for various species of rickettsiae which differ considerably in their properties demonstrates the close bonds which exist between rickettsiae and arthropods, and indicates phylogenetic relationships between rickettsial species. If the behavior of rickettsiae in lice is used as a taxonomic base, *R. quintana* and *R. tsutsugamushi* will occupy exceptional positions. With *R. quintana* this results from its extracellular growth in the lumen of stomach and its requirement of human lice, while lack of ability to reproduce in the stomach justifies the exception of *R. tsutsugamushi*. The behavior of *C. burnetii*—abnormal development and the occasional invasion of organs—

must be considered exceptional, too. The behavior of the remaining rickettsiae is generally in accordance with the classification of rickettsiae transmitted by ticks and insects. This classification indicates also the close relationships between *R. prowazekii* and *R. typhi*.

The biological relationships which exist between lice and rickettsiae are demonstrated by the ability of rickettsiae to survive for a considerable time and to multiply intensively in the stomach of lice. Evidently *R. quintana* is best adapted to lice, since the lice will not be harmed in any way by the infection. This is partly attributable to the extracellular reproduction of this agent. It is difficult to decide whether this relationship must be considered a primary or secondary adaptation. Furthermore, there are natural relationships between lice and *R. prowazekii* and *R. typhi*. Obviously the host-parasite association is not so old as that between rickettsiae and fleas, for rickettsial parasitemia involves considerable injury of the lice [Mooser (41)]. Nevertheless, the adaptation is sufficient to ensure continuity of the species. The rickettsiae multiply intensively before the host becomes lethally injured. Sometimes there is even a balance between host and parasite, i.e., infected lice may harbor rickettsiae for life. Furthermore, large numbers of rickettsiae are voided with the feces where they may survive for several weeks, although rickettsiae are normally very sensitive [Chao (11); Starzyk (75); Weyer (90)]. *R. quintana* and *R. prowazekii* require lice and humans for their growth, the reservoir being formed by the latter; animal reservoirs do not exist. Late relapses of epidemic typhus (Brill-Zinsser disease) may lead to an infection of lice and thus to reappearance of the disease after very long intervals [Mooser (42); Murray & Snyder (49); Weyer & Hornbostel (91)].

Ticks must be considered the original hosts of rickettsiae. These microbial agents will grow in ticks even if they are now normally transmitted by fleas and lice, which are probably secondary if not tertiary hosts. We do not know when in the course of evolution the group of rickettsiae (originally uniform, as it must be presumed) was split and divergent development began. The migrations of animals harboring rickettsiae after separation of the continents and the transition of these agents to other blood-sucking arthropods after a passage of warm-blooded hosts may have had a bearing on this phenomenon.

RELATIONSHIPS BETWEEN LICE AND SPIROCHETES

The biological relationships between lice and the agents of relapsing fever are clearly recognizable and must be considered perfect. Originally it had been assumed that lice were capable of transmitting only the agent of epidemic relapsing fever, *Borrelia recurrentis* (Lebert), but now it is a well-established fact that most species of *Borrelia* will survive and multiply in lice. Basic investigations on the behavior of *Borrelia* in lice were carried out years ago by Nicolle & Blanc (53) and Nicolle, Blaisot & Conseil (54). Further valuable observations were made by Chapcheff (12), Chung & Feng (13), Sparrow (72, 73, 74), Toyoda (78), Wolman & Wolman (94), and others. Experiments on natural inoculation—by feeding lice on infected persons or animals—were supported by investigations on artificial inoculation [Baltazard

et al. (4); Mooser & Weyer (46); Sparrow (72); Weyer & Mooser (92)].

Some time after inoculation, the spirochetes will be abundant in the hemolymph of the lice, larvae as well as adults, where they readily multiply. They remain confined to the hemolymph and will not invade any organs, including sexual organs and salivary glands. Thus transmission by biting or any kind of transovarial transfer is impossible. The spirochetes can only leave their hosts together with the hemolymph—if this is lost after injury, for instance—and may then invade humans. A louse once infected will harbor spirochetes for life.

The percentage of lice which will become infected depends both on the species of *Borrelia* and on the amount of spirochetes ingested. The spirochetes reach the hemolymph, where their reproduction takes place, after active penetration of the stomach wall, but only few of them will succeed. With *B. recurrentis*, Chung & Feng (13) estimated that maximally 1 to 5 per cent of the ingested parasites will reach the hemolymph. Some of the spirochetes are possibly voided with the feces while the louse is feeding, the majority, however, will die within a few hours in the arthropod's stomach. Only those spirochetes which succeed in reaching the hemolymph after passing through the stomach wall are of practical importance. Until recently the mode of this penetration was rather obscure. Most investigators believed that the spirochetes undergo a cyclic development in lice, during which they will disintegrate when passing the stomach wall, thus forming an invisible or hardly demonstrable stage, to reappear as "metacyclic spirochetes" in the hemolymph after 5 to 10 days. This assumption was maintained by Heisch (29, 30), Heisch & Garnham (31), Nicolle & Anderson (52), Nicolle & Lebaillly (55), Sergent & Foley (66), and others. Their theory was mainly supported by the fact that no spirochetes were detectable in lice during the first days following the infection.

This hypothesis, which became questionable by the discovery that lice are infective during the "invisible stage," was based on inadequate methods of investigation [Mooser (43)]. When thoroughly examined, spirochetes will sporadically be found in the hemolymph of lice a few hours after the inoculation, i.e., those which succeeded in passing the stomach wall. This phenomenon had already been described by Toyoda (78) and was later confirmed by Wolman & Wolman (94) and others. The spirochetes remain in lice without changing their shape, stainability, mobility, and virulence. Their number is constantly growing by fission. Evidently their propagation rate increases after 5 to 7 days, the maximum being reached after approximately 10 to 12 days. The occurrence of the so-called metacyclic stage is actually the phase during which the spirochetes are so numerous in the hemolymph that they can be detected by less intensive methods of investigation. The appearance of this stage, as well as the number of positive lice, is dependent on the number of spirochetes which succeeded in penetrating the alimentary canal and on environmental conditions. These findings readily explain the contradictory experimental results. In cases of severe spirochetemia or inoculation of large doses, all lice will become positive.

The hemolymph of lice is a suitable medium not only for *B. recurrentis* but also for other species, which are naturally harbored by ticks. Baltazard *et al.* (4) were able to transfer the following species of *Borrelia* experimentally to lice: *B. microti* (Rafyi), *B. turicatae* (Brumpt), *B. merionesi* (Blanc & Maurice), *B. parkeri* (Davis), *B. hermsii* (Davis), *B. hispanica* (de Buen), and *B. anserina* (Sakharoff). Only *Borrelia persica* (Dschunkowsky) proved to be an exception [Adler & Ashbel (1)]. Further successful transmission experiments were carried out with *Borrelia crocidurae* (Leger), *B. novyi* (Schellack), and *B. dipodilli* (Heisch). Particularly interesting are observations by Heisch & Garnham (31) and Heisch (29), who were able to demonstrate propagation of *Borrelia duttonii* (Novy and Knapp) in lice. Mooser & Weyer (46) maintained three strains of *B. duttonii* from Tanganyika continuously in body lice up to seven months without the spirochetes changing their properties. After rectal inoculation with diluted mouse blood 25 to 37.5 per cent of the lice became positive, while up to 47.8 per cent of the lice responded positively after natural inoculation by feeding on mice. A strain of *Borrelia turicatae* (Brumpt) proved to be much less adapted to lice [Weyer & Mooser (92)]. On the other hand, a strain (a variety of *B. crocidurae*) which had been isolated from the small subspecies of *Ornithodoros erraticus* (Lucas), yielded 100 per cent positive lice after their feeding on mice.

Intracoelomic inoculation of spirochetes directly into the hemolymph of lice is followed by immediate reproduction. Thus, this method is particularly useful for maintaining those strains under laboratory conditions for which procuring of suitable animal hosts is difficult. It also represents a simple means of testing the propagation rate of various spirochetes in lice. Applying this technique, Geigy, Mooser & Weyer (24) tested several East African strains of *B. duttonii* from *Ornithodoros moubata* (Murray) which had become avirulent for mice. These strains did not even multiply after direct inoculation into the hemolymph, though their viability and transferability to ticks remained unchanged for several weeks in the hemolymph.

According to the data available, which are rather incomplete, spirochetes—at least *B. recurrentis* and *B. duttonii*—are capable of reproduction not only in body lice but also in head lice. Corresponding observations on crab lice are still lacking, as are systematic or well-established studies on the behavior of spirochetes in animal lice, especially rat lice. Experiments have been carried out only on monkey lice, which also proved susceptible. On the other hand, *B. duttonii* did not multiply in pig lice, neither after rectal nor after intracoelomic inoculation [Mooser & Weyer (46)].

The biological relationships between lice and spirochetes are revealed by the spirochetes' ability to survive in the stomach of the louse, to penetrate the stomach wall, to invade the hemolymph, and to multiply here without being affected themselves or harming their hosts. The better adaptation of *B. recurrentis* is apparently demonstrated by the fact that these spirochetes will reach the hemolymph more easily than other species. This ability to reach the hemolymph within a certain time seems to be a distinctive characteristic of the species of *Borrelia* which have so far been investigated. Only

one species, *B. persica*, is known to be lacking this ability. On the other hand, Heisch (29) was able to prove that body lice may be responsible for limited outbreaks of relapsing fever caused by *B. duttonii* in East Africa.

Penetration of the stomach wall is a decisive prerequisite to the growth of spirochetes and to their fate. We do not know as yet the factors governing this process. It is surprising how limited the chances are that spirochetes will reach a new warm-blooded host, as they are not voided with the feces, do not invade any organs, and are not transovarially transmitted. Their only way of transmission is via the hemolymph after injury of the lice, and it may be purposive that the spirochetes are most abundant in the hemolymph of the legs, which break easily. This single mode of transmission is a significant distinction between the behavior of louse-borne and tick-borne spirochetes. Louse-born spirochetes compensate for their small chance of finding a new host by their rapid propagation in lice, which will remain infected for life, and the large number of suitable hosts they find in cases of lousiness.

Concerning the origin of louse-borne relapsing fever, Nicolle and his collaborators offered the theory—which has been neither confirmed nor refuted—that this disease gradually developed out of tick-borne relapsing fever through adaptation of the agents to the cycle: man → louse → man [cf. Nicolle & Anderson (51)]. This view is supported by the fact that tick spirochetes will readily multiply in body lice. It must be emphasized, however, that *B. recurrentis* definitely lost its ability to grow in ticks. Lice must be considered recent hosts as compared to ticks.

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